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Parenteral Formulations Based on Albumin Particulate Technology

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ABSTRACT – Over the years, nanoparticle drug delivery systems have demonstrated versatile potentials in biological, medical and pharmaceutical applications. In the pharmaceutical industry nanotechnology research has mainly focused on providing controlled drug release, targeting their delivery to specific organs, and developing parenteral formulations for poorly water soluble drugs to improve their bioavailability. Achievement in polymer industry has generated numerous polymers applicable to designing nanoparticles. From viewpoints of product development, a nanocarrier material should meet requirements for biodegradability, biocompatibility, availability, and regulatory approval crieteria. Albumin is indeed a material that fulfills such requirements. Also, the commercialization of a first albumin-bound paclitaxel nanoparticle product (Abraxane™) has sparked renewed interests in the application of albumin in the development of nanoparticle formulations. This paper reviews the intrinsic properties of albumin, its suitability as a nanocarrier material, and albumin-based parenteral formulation approaches. Particularly discussed in detail are albumin-based particulate injectables such as Abraxane™. Information on key roles of albumin in the nab™ technology and representative manufacturing processes of albumin particulate products are provided. It is likely that albumin-based particulate technology would extend its applications in delivering drugs, polypeptides, proteins, vaccines, nucleic acids, and genes.

Key words – albumin, nanoparticles, nanotechnology, injectables, parenteral formulation

Recently, significant efforts have been devoted to the advancement of nanotechnology in the pharmaceutical industry since it can provide a suitable means of site-specific, controlled delivery of small or large molecular weight bioactive agents. Nanoparticulate drug delivery systems are nanometer-sized carriers. They possess various morphologies including nanospheres, nanocapsules, nanomicelles, nanostructured lipid carriers, nanoliposomes, nanodrugs, nanoplexes, and so on (Jung et al., 2000; Reis et al., 2006; Vauthier and Couvreur, 2000; Howard and Kjems, 2007). In general, nanoparticles are usually matrix particles having spherical morphology, and they are termed nanospheres. Nanocapsules differ from nanospheres in that the former consist of a solid material polymeric shell surrounding a core which is either liquid or semisolid. Nano-sized polyelectrolyte complexes are called nanoplexes. Figure 1 illustrates various morphology of nanoparticles.

Not only drugs can be loaded into biopolymeric nanoparticles, but also bulky drug substances themselves are comminuted to nanometer-sized particles. At first, nanotechnology in the pharmaceutical industry has boomed toward poorly water soluble drugs. The pharmaceutical development of a poorly water soluble drug substance is often encountered with bioavailability problems. Poorly water soluble drugs pass through the gastrointestinal tract before being absorbed into systemic circulation. Administration of drugs with poor water solubility in the form of nanoparticles helps increase their bioavailability, since bioavailability is proportional to the surface area. Therefore, reductions in their particle size result in increased bioavailability (Busch-vishniac et al., 1996). For a safety reason, there is concern on the intravenous administration of poorly water-soluble drugs. Therefore, nanoparticulate formulations that do not count on organic solvents and surfactants would be ideal for their intravenous administration (Damascelli et al., 2001; Ibrahim et al., 2002).

In cases when drugs are loaded into nanoparticles, some are physically entrapped across the polymeric matrix, or the others are adsorbed on the surface of nanoparticles. They can also be first covalently bound to nanoparticle-forming materials. Nanoparticles have several intrinsic properties as follows: they pass through the smallest capillary vessels because of their submicron size and avoid rapid clearance by the reticuloendothelial system in the body. Subsequently, their duration in blood stream is prolonged to great extents; nanoaparticles can penetrate cells and tissue gap to arrive at target organs such as the liver, the spleen, the lung, and etc.; and nanoparticles can provide controlled drug release, in case when their properties are manipulated in terms of biodegradability, pH, ion and/or temperature sensibility. Therefore, various benefits can be expected

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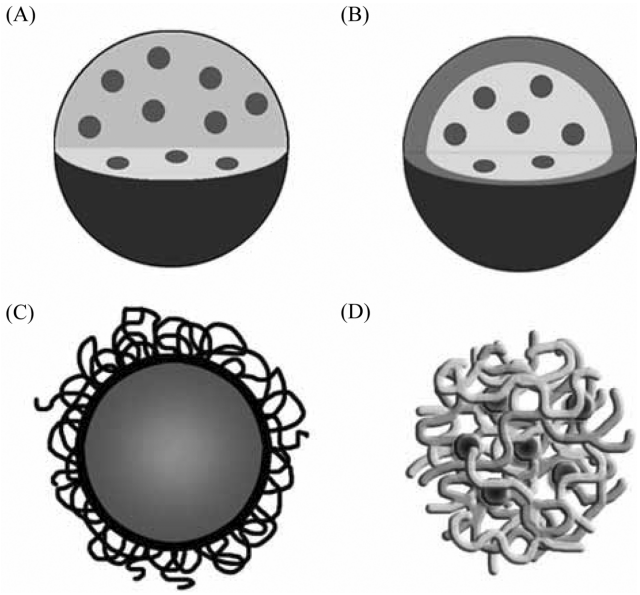

Figure 1. Schematic representation of some typical nanoparticles: (A) a nanosphere, (B) a nanocapsule, (C) a nanodrug particle associated with a surface modifier, and (D) a nanoplex.

Table I. *Use of Human Serum Albumin as an Inactive Ingredient in FDA-approved Drug Products*[a]

Inactive ingredient	Route	Dosage form	Maximum potency
Albumin, aggregated	IV	injection	0.15%
Albumin, colloidal	IV	powder for injection	0.10%
Albumin, human	IV	injection	1.00%
Albumin, human	IV	power for injection	1.00%
Albumin, human	IV (infusion)	injection	1.00%
Albumin, human	IV (infusion)	powder for injection	80.00%
Albumin, human	SC	injection	0.10%
Albumins	Oral	film-coated tablet	4.50 mg
Alumin microspheres	IV	injection	0.50%

[a]The result of inactive ingredient search of albumin for the FDA-approved drug products in the United States (The Inactive Ingredient Database of the US FDA was utilized for search).

from nanoparticulate drug delivery systems. In general, fragile molecules are better preserved from enzymatic degradation in the body when they are entrapped in the nanocarrier (Lambert et al., 2000; Toub et al., 2006). Nanoparticle-forming materials can be chemically and/or physically modified to modulate their interactions with blood proteins or mucosa, thereby leading to manipulation of their in vivo performance (Torchilin and Trubetskoy, 1995; Couvreur and Vauhtier, 2006; Avgoustakis, 2004; Mosqueira et al., 2001). When anticancer drugs are formulated into nanoparticles, a passive tumor targeting is often possible due to the so-called enhanced permeation and retention (EPR) effect (Maeda et al., 2000). Furthermore, nanocarrier systems help overcome multidrug resistance in various cancers (Barraud et al., 2005). As a consequence, nanoparticles would be well tolerated without any serious side-effects, thereby contributing to improved drug safety and efficacy.

Rationale for Selecting Albumin as Carrier Protein

Advancement in polymer chemistry today has generated numerous polymers. As a result, various macromolecular substances such as synthetic and natural polymers are being used to design nanoparticles for drug delivery (Kreuter, 2004). However, in order to use them in developing parenteral products, several factors should be taken into consideration. First of all, a polymer candidate should be biodegradable so that it is completely eliminated from the body. This would eliminate any risk associated with accumulation in the body. A carrier itself and its degradation products should be non-toxic and non-immunogenic, thereby assuring biocompatibility. To develop a commercial product, the polymer candidate should be readily available at a comparatively inexpensive cost. Finally, a nanocarrier material should present no obstacles in receiving regulatory approval from health authorities. Albumin, having ability to fulfill all these requirements, is a nanocarrier material of choice. At present, human serum albumin is used in many commercial drug products. Table 1 shows the result of inactive ingredient search of albumin for approved drug products in the United States.

It is worthwhile to look over major physicochemical properties of albumin, in order to understand the potential of albumin as a drug carrier. An excellent review on the characteristics of albumin is found in literature (Peters, 1985) Albumin is a major soluble protein of the circulating system with a blood concentration of about 50 mg/mL. Albumin, having molecular weight of 69 kDa, consists of 585 amino acid residues. Albumin is highly polar−Such a property can be easily projected from its amino acid composition having 100 negative and 82 positive charges. Albumin has a free sulfhydryl group in the position 34, and total 17 disulfide bonds are involved in deciding its tertiary structure. In circulating plasma, 30% of this free sulfhydryl in Cys34 is oxidized by cysteine and glutathione (Carter and Ho, 1994). At a normal condition, the disulfide bonds of albumin are well shielded from the solvent. They are not accessible to reducing agents in a neutral pH range, but

become exposed at acidic or alkaline pHs. Its isoelectric point (pI) is around pH 4.8, when it contains fatty acids. This means that albumin itself carries a net negative charge at a neutral pH. In general, albumin has extraordinary stability, such that it shows strong resistance toward denaturation. At acidic pHs, albumin expands and elongates, but it returns to native conformation when pH shifts to neutrality. At alkaline pHs, albumin is also relatively unharmed but vulnerable to deamidation or disulfide interchange. In addition, albumin has amphiphilic nature with flexible conformation. These features endow albumin with ability to bind with various ligands having different hydrophilicity/hydrophobicity. There have been numerous reports and reviews dealing with the binding of various ligands to albumin, and an excellent review was published in 1981 (Kragh-Hansen, 1981). Albumin–ligand bindings can be studied by using techniques such as fluorescence, equilibrium dialysis, dynamic dialysis, ultrafiltration, or difference spectrophotometry.

In fact, human serum albumin (HSA) has multiple hydrophobic binding sites and binds a diverse set of drugs, especially neutral and negatively charged hydrophobic compounds. Two high affinity binding sites were reported in subdomains IIA and IIIA of HSA, which are highly elongated hydrophobic pockets with charged lysine and arginine residues near the surface. They function as attachment points for polar ligand features (Fehske et al., 1981; Vorum, 1999; Curry et al., 1998; Sugio et al., 1999; He and Carter, 1992; Carter and Ho, 1994). Due to the fact that a large number of drugs bind to albumin, their bindings have often been considered nonspecific. However, in some cases ligand-albumin binding bears specificity and their binding accompanies the formation of a covalent bond. For example, the residue of cysteine 34 can react with a ligand with a sulfhydryl group and cationic metals of Ag^{+} and Hg^{++}. Glycosylation occurs between glucose and lysine residues in the albumin molecule. Also, aspirin is covalently bound to a lysine residue of HSA. Furthermore, the β-lactam ring of several penicillins reacts with lysine and histidine residues of albumin. In most cases, the ligand-binding affinity of albumin is superior to that of other proteins. These traits suggest that albumin differs from typical proteins and why it finds versatile applications as a drug carrier.

Drug Nanoparticles Preparation

Some patents disclose how to prepare drug nanoparticles and describe several methods for modifying their surface properties. In this practice, albumin is used as a surface modifier that can associate with drug nanoparticles to change their physical and biopharmaceutical properties. Therefore, general approaches used to prepare drug nanoparticles will be first discussed here. Drug substances having bad water solubility often show poor bioavailability, due to their dissolution-related problems. Their bioavailability is often proportional to the total surface area of the drug substances. Therefore, administration of such drug substances in a nano-sized form results in marked improvement in their bioavailability. This is one reason why poor water solubility of many drug substances has propelled the pharmaceutical industry to develop nanoparticle technology. As a result, a number of poorly water soluble drugs have been formulated into nanoparticle dosage forms for oral and parenteral administration. The former NanoSystems L.L.C. (Collegeville, PA, USA) was one of the pioneering companies specialized in this area and was assignee of a series of relevant patents. The following are general approaches used to prepare drug nanoparticles.

Grinding

In this practice, a drug substance is comminuted in the presence of a surface active agent which is termed a surface modifier. As an alternative, a surface active agent is allowed to adsorb on drug nanoparticles after the comminution process is complete (Liversidge et al., 1995; Liversidge et al., 1992; De Garavilla et al., 1998). The function of a surface modifier is to prevent aggregation or flocculation of drug nanoparticles. For instance, an anticancer drug powders with less than 100 μm in size are dispersed in a liquid dispersion medium containing a surface modifier. If the drug particle size is greater than 100 μm, they are treated with a conventional milling method (e.g., airjet or fragmentation milling) to decrease their size to less than 100 μm. Mechanical means (e.g., ball/bead/vibratory/planetary milling) are then applied in the presence of a grinding medium to make drug particle size less than 1000 nm. An example of the grinding medium is zirconium oxide beads. Alternatively, the above dispersion can be ultrasonicated to prepare nano-sized particles. The dispersion of the surface modified anticancer drug nanoparticles can be coated onto sugar spheres by spray drying, or is concentrated by diafiltration or evaporation. The following is an example of a grinding method to prepare piposulfan nanoparticles: Piposulfan was milled in a mixture of 0.33% polyoxyethylene sorbitan monooleate, Tween 80, and 0.67% sorbitan monooleate, Span 80 using 1 mm zirconium oxide beads for about 96 hr. This treatment resulted producing drug nanoparticles with 240 nm in diameter. The final piposulfan concentration in the suspension was 10 mg/mL. A milling process to prepare a coarse suspension of piposulfan was as follows: 300 mg of the drug

was added to a dispersion medium consisting of 60 mL of 1 mm zirconium oxide beads and 30 mL of 1% Tween 80/Span 80 (1:2 ratio) solution. The above system was milled on a roller mill for 96 hr. The slurry was separated from the media at the end of the milling time. The final pH of the formulation was 6. Camptothecin nanoparticulate was also prepared following a similar grinding method. To do so, 0.35 g of Tetronic 908 and 0.35 g of camptothecin were added to 60 mL of precleaned zirconium oxide beads. Being combined with 35 mL of water, the mixture was subject to roller milling for 7 days. The resultant particles had an average particle size of 240 nm.

Instead of milling, microfluidization also can be used to provide comminution action. Water insoluble, crystalline drug particles having a surface modifier on their surface are dispersed in a liquid medium. The coarse dispersion medium is comminuted by use of a microfluidizer that displays shear, impact and cavitation forces. This treatment can reduce the size of drug particles to less than 400 nm. In fact, a number of literature and patents describe the use of microfluidizers to prepare emulsions, liposomes, and/or particulate suspensions (Meeh and Cacheris, 1994; Grunewalder and Voelker, 1993; Gregoriadis et al., 1990; Doegito et al., 1994; Talsma et al., 1989; Lidgate et al., 1990; Bodmeier and Chen, 1990; Koosha and Muller, 1988).

A variety of surface modifiers are proposed in the patents mentioned above. Examples are various polymers, oligomers, natural products and surfactants. Particularly, the preferred surface modifiers are polyvinylpyrrolidone, polaxomers, poloxamines, dextran, lecithin, sodium lauryl sulfate, Tweens, Spans, polyvinyl alcohol, gum acacia, hexyldecyl trimethyl ammonium chloride, and bovine serum albumin. Some distinguished features are found with the drug nanoparticles associated with the surface modifiers. First of all, surface modifiers are physically adsorbed on the surface of drug particles, and there are no covalent bonds between drug particles and surface modifiers. Also, there are no intermolecular crosslinkages among the surface modifiers adsorbed on the surface of drug particles. Finally, the physical state of the nano-sized drug particles, after the preparation process, is present in one or more discrete crystalline phases.

Solvent precipitation

Solvent precipitation is one method used to prepare uniformly sized particles from water insoluble drugs or other organic compounds (Violanto, 1989). First, a water insoluble drug having little aqueous solubility is dissolved in a water miscible organic solvent (e.g., dimethyl sulfoxide, dimethyl formamide, N,N'-dimethyl acetamide, phenol, or isopropanol). The solution is then diluted with an aqueous precipitation liquid (e.g., water, a solution of a mineral salt, or a surfactant solution. Surfactant examples include polyvinyl pyrrolidone, human serum albumin, Pluronic F-68, and gelatin). The infusion of the aqueous precipitating liquid triggers the precipitation of non-aggregated drug particles with the size range of 0.01 to 5 μm. The particles are then separated from the organic solvent. Important process parameters affecting the size distribution of drug particles include temperature, ratio and volume of non-solvent to organic solvent, infusion rate, stirring rate, and the time interval between onset and completion of precipitation. For instance, the particle size is directly related to the solution temperature and is inproportional to infusion rate. A very short time interval between onset and completion of precipitation produces uniformly sized particles, while a long time interval results in the formation of drug particles with a broad size distribution. It is possible to produce drug particles with a desired size by carefully manipulating the relevant parameters. At the end of the conventional solvent precipitation-based manufacturing process, drug particles tend to exist in a noncrystalline, amorphous state. In addition, the solvent precipitation techniques provide particles contaminated with organic solvents. Organic solvents are often toxic and can be very difficult, if not impossible, to reduce their residual amounts in the nanoparticles to pharmaceutically acceptable levels.

Microprecipitation

In the practice of this microprecipitation technique, a drug substance is dissolved in an aqueous base solution containing a nontoxic water miscible solvent (e.g., methanol, ethanol, n-propanol, or isopropanol) (Bagchi et al., 1997). A surface modifier, such as Aerosol A012, is added to the solution, which is then immediately neutralized with an appropriate acid solution. Salts and solvent are removed by either diafiltration or dialysis. The colloidal dispersion can be concentrated by conventional means such as evaporation. The surface modifier adsorbing on the surface of drug nanoparticles help keep them from congregation or flocculation. Therefore, their size remains unchanged upon storage.

Spray drying

Spray drying is used to produce fine drug particles. For example, the size of commercially available paclitaxel powders is greater than 20 μm. Therefore, fine particles of paclitaxel less than 5 μm, preferably 1 μm, were prepared by spray drying (Desai et al., 1995). In this manufacturing process, paclitaxel is first dissolved in a volatile organic solvent such as

ethanol. The solution is then passed through an ultrasonic nozzle to form fine ethanolic solution in which taxol is dissolved. Evaporation of the ethanol inside the spray drier leads to preparation of fine paclitaxel particles. The particle size of paclitaxel can be adjusted by changing the paclitaxel concentration in ethanol, the flow rate of the ethanolic solution through the nozzle, and the sonication power.

Albumin Conjugates

A number of studies show that bioactive materials are fused or conjugated to albumin itself or albumin particles, in order to reduce dosing frequency by extending their half-lives. Examples include insulin, glucagon-like peptide 1, albuglutide, human interferon, thioredoxin as a redox-active protein with anti-inflammatory effects, doxorubicin, apolipoprotein, opoids, and the like (Tomkin, 2009; Rustgi, 2009; Ikuta et al., 2010; Ohkawa et al., 1993; Michaelis et al., 2006; Holmes et al., 2000; Jette et al., 2005; Thibaudeau et al., 2005; Eatock et al., 1999). These approaches generate new chemical entities that might require a lengthy process for obtaining product approval from regulatory authorities (Duncan, 2003).

Albumin Nano/Microparticles

Albumin nano/microspheres have been considered promising drug delivery systems for a long time. Even though some reports introduce denatured albumin nano/microparticles, majority of relevant studies focus on crosslinked albumin nano/microspheres. Various methods have been developed to prepare them, and the following are some representative approaches.

Suspension crosslinking

Suspension crosslinking is a well known method for preparing protein microspheres. In the practice of this manufacturing process, an aqueous protein solution is added to an immiscible solvent such as oil. Emulsification is performed to make tiny aqueous droplets dispersed in the oil phase. The emulsion is subject to heating above 80°C or crosslinking with glutaraldehyde, or combination of the two. Various approaches based on this principle are well described in literature (Arshady, 1990). Solid albumin microspheres produced by mild chemical crosslinking were introduced by Dr. Sokoloski's research group, so as to explore their potential as a parenteral drug delivery system (Lee et al., 1981). In their study progesterone was suspended in 0.8 ml of pH 7.5 phosphate buffer containing 0.1% sodium lauryl sulfate. Albumin (200 mg) was dissolved in the suspension, to which 0.2 mL of glutaraldehyde was added (the final glutaraldehyde concentration was 1%). The mixture was rapidly emulsified in 100 mL of a continuous phase consisting of petroleum ether and corn oil at a 4:1 ratio. After 1hr-stirring, microspheres were collected and dried in a vacuum desiccator. Prepared in this manner were microspheres with 5 to 30% progesterone payloads. Their size ranged between 100 to 200 μm. In vivo release experiments using rabbits displayed that the crosslinked microspheres provided sustained progesterone release for about 20 days. In vitro study showed that the crosslinked albumin microspheres were vulnerable to chymotrypsin digestion, suggesting their biodegradability. The same research group scrutinized the effects of rate and extent of crosslinking on microsphere properties (Sheu et al., 1986; Sheu and Sokoloski, 1986; Sheu and Soloski, 1991). When glutaraldehyde is used as a crosslinking agent, a reaction involving oligomerization across ε-amino groups of lysine residues in albumin occurs spontaneously and rapidly. Therefore, an aqueous phase containing a drug, albumin and glutaraldehyde should be rapidly emulsified in a continuous oil phase before crosslinking occurs to a considerable extent. Thus, factors affecting the emulsion droplet size and the reaction rate determine the size of albumin microspheres. Examples of such variables are: the apparatus design and capacity, the rate of emulsification/stirring, the density and viscosity of the internal aqueous and external oil phase, and the interfacial tension, pH and buffer concentration of the aqueous phase, drug loading, albumin and glutaraldehyde concentrations, and the like. However, there has been concern on the use of a crosslinking agent which is toxic to humans.

Coacervation

A coacervation method is applied to prepare albumin microspheres having the size range of 0.1 to 5 μm. Ethanol or isopropyl alcohol is used as a coacervation agent which was added to an aqueous albumin solution. A similar technique has been developed to prepare albumin nanoparticles by adding acetone to an aqueous albumin solution and heating the dispersion (Chen et al., 1994). In general, coacervation or controlled desolvation methods are simpler to operate than suspension crosslinking methods. Also, the particulates prepared by coacervation are free of toxicity problems arising from a crosslinking agent. However, a major drawback is related to stability: particulates prepared by coacervation tend to be unstable so as to form larger aggregates on storage. In addition, it is hard to prepare nano-sized particles of less than 200 nm which is the preferred size for intravenous administration. A conventional coacervation method was modified to

improve the formation of nanoparticles and their stability (Coombes et al., 2003; Coombes et al., 2001). This method was developed by observing that when the pH of an aqueous albumin solution was lowered to about 4.4 to 4.7 with α-hydroxy acid (e.g. lactic acid), albumin precipitated rapidly and extensively. The resulting precipitates in the form of nanoparticles had exceptional stability. More specifically, albumin nanoparticles were prepared by mixing 2 mL of 2% aqueous albumin solution with 20 μL of 85% lactic acid solution. Acetone (5.2 mL) acting as a coacervating agent was added to the solution, which was stirred overnight. The evaporation of acetone led to production of albumin nanoparticles in the size of 200 nm. The nanoparticles are less toxic than the albumin nanoparticles produced by use of a crosslinking agent such as glutaraldehyde. Another interesting feature is that this process does not use any surfactant, which contrasts to conventional emulsion-based manufacturing processes.

Desolvation followed by crosslinking

Human serum albumin (HSA) nanoparticles are considered promising drug carrier systems, especially for cystostatics. Their binding to HSA nanoparticles may diminish their toxicity, optimize their body distribution and/or overcome multidrug resistance. For example, Dr Langer's research group reported how to prepare doxorubicin-loaded albumin nanoparticles (Dreis et al., 2007). Doxorubicin was loaded to albumin nanoparticles either by adsorption to the nanoparticle surface or by incorporation into the nanoparticle matrix. Both approaches resulted in the formation of albumin nanoparticles having the size range of 150 to 500 nm. At the same time, the drug loading efficiency exceeded with 70%. The influence of the nanoparticles on cell viability was investigated in two different neuroblastoma cell lines. The anti-cancer effects of the drug-loaded nanoparticles were increased, compared to a doxorubicin solution. For these studies, they prepared albumin nanoparticles by desolvation followed by cross-linking (Weber et al., 2000; Langer et al., 2003). A typical manufacturing process of their nanoparticles could be summarized as follows. Doxorubicin and albumin were dissolved in 2 mL of water, and the solution was stirred for 2 hr to achieve the adsorption of doxorubicin to the protein (the solution pH was kept at 6.5 or 8.2). An adequate amount of ethanol was added to the solution at a rate of 1 ml/min using a tubing pump. The appearing nanoparticles were stabilized by the addition of an aqueous 8% glutaraldehyde solution (1.175 μL/mg HSA). The crosslinking step was performed under constant stirring of the suspension over 24 hr. After centrifugation, the nanoparticles were redispersed in purified water by ultrasonication in order to eliminate excipients such as ethanol and glutaraldehyde. An interesting parameter that affected the loading efficiency of doxorubicin was the solution pH–Changing the solution pH from 8.2 to 6.5 helped improve doxorubicin loading efficiency. The result suggests that a pH-dependent conformation change of albumin affect its binding affinity toward doxorubicin.

Emulsification

A number of studies report an emulsification-based process to make albumin nanoparticles (Müller et al., 1996; Yang et al., 2007). For instance, Yang et al. prepared their nanoparticles by the following method: An alkaline aqueous solution with albumin and a drug were homogenized in castor oil containing 2% Span 80 at room temperature. The resultant emulsion was then transferred dropwise to another castor oil of which temperature was set at 140°C, and the resulting mixture was stirred for 15 min. This heat treatment led to stabilization of albumin nanoparticles. Petroleum ether was then used to wash albumin nanoparticles off castor oil and the surfactant. Their study demonstrated that albumin particle size was affected by process parameters such as aqueous albumin concentration, emulsification time, the ratio of aqueous phase to oil phase, emulsion dropping rate, heat stabilization temperature, and heat treatment time, and the like. A major disadvantage of this emulsion-based method may arise from the use of an oily phase. It is hard to completely remove the oily residue from nanoparticles.

Spray-drying method

Albumin microparticles in the size range of 2 to 10 μm was prepared by spray drying followed by heat treatment at 100 ~150°C for 6~24 hr (Pavanetto et al., 1994). One advantage of spray drying is that the method does not use organic solvents so that there is no concern in the issues of residual solvents in albumin particles.

Albumin-bound Paclitaxel Nanoparticles (nab™-paclitaxel; Abraxane®)

The first albumin-bound nanoparticle product (Abraxane®) was approved by the FDA in 2005. The nanoparticles are in the size of 130 nm and consist of paclitaxel and albumin. Abraxane® is presented as a lyophilized cake made of 900 mg of human serum albumin and 100 mg of paclitaxel. Because of albumin surrounding the solid drug core, the nanoparticles are negatively charged and stabilized by electrostatic repulsion against aggregation. This drug product is not based on the Cremophor EL/ethanol formulation. Being reconstituted with 20

ml of 0.9% NaCl solution, this product is given intravenously to patients at the dose of 260 mg/m^2 over 30 minutes every 3 weeks. It is claimed that the albumin-bound nanoparticles offer several benefits with regard to drug safety and efficacy, compared to a standard paclitaxel therapy (Gradishar et al., 2005; Desai et al., 2006). The nab^{TM}-paclitaxel formulation helps reduce the risk of hypersensitivity reactions, does not require premedication and is given over a shorter period (30 min) without using a special intravenous tubing. The following sections discuss key aspects of the albumin-bound paclitaxel nanoparticles.

Issues associated with the solvent-based injectable formulations of taxanes

Taxanes such as paclitaxel and docetaxel have poor solubility in water. This property hampered their pharmaceutical development for a long time (ten Tije et al., 2003). Concerted efforts led to the development of a drug product using polyethoxylated castor oil (Cremophor EL) and ethanol as paclitaxel solubilizers (Taxol®) (Gelderblom et al., 2001). In case of Taxotere®, polysorbate 80 and ethanol are used as solubilizers of docetaxel. A standard paclitaxel therapy requires pretreatment with corticosteroids and antihistamines to reduce the risk of hypersensitivity reactions. Sometimes, Cremophor EL can cause neutropenia, and prolonged peripheral neuropathy, which may be associated with axonal degeneration (Hawkins et al., 2008; Dye and Watkins, 1980; Gelderblom et al., 2001; Weiss et al., 1990; Kloover et al., 2004; Volcheck and Van Dellen, 1998; Gianni et al., 1995). In cases when polysorbate 80 is used in formulations, there are issues associated with hypersensitivity reactions, as well as sensory and motor neuropathies (Gligorov and Lotz, 2004; van Zuylen et al., 2001). Cumulative fluid retention is a side effect unique to a docetaxel product, which is attributed to polysorbate 80 that can alter membrane fluidity.

Role of albumin in the nab^{TM} technology

Compared with other carrier proteins, albumin has several unique features that make it a particularly attractive carrier of anticancer drugs. Albumin is a natural carrier of a variety of hydrophobic molecules, and their binding to albumin is reversible so that they become unbound at the cell surface (Purcell et al., 2000; Paal et al., 2001). In fact, several studies reported that more than 85% of paclitaxel binds to albumin (Paál et al., 2001; Purcell et al., 2000; Altmayer et al., 1995; Garrido et al., 1994; Kumar et al., 1993). Also, a same binding pattern was observed with healthy volunteers and cancer patients. The degree of binding was found to be concentration independent, indicating binding occurs nonspecifically. All these results indicate that there is a strong binding affinity between albumin and paclitaxel.

Another prominent feature of albumin is that it takes part in the active transport of paclitaxel into a tumor site by an albumin receptor. It is proposed that albumin facilitates endothelial transcytosis of unbound and albumin-bound plasma constituents into the extravascular space. This process is initiated by binding of albumin to a cell surface, 60-kDa glycoprotein (gp60) receptor (albondin). This results in binding of gp60 with an intracellular protein (caveolin-1) and subsequent invagination of the cell membrane to form the so-called caveolae vesicles (John et al., 2003; Minshall et al., 2002). An interesting preclinical study demonstrated that endothelial binding of paclitaxel increased 9.9-fold and transport of paclitaxel across the endothelial cell monolayer increased 4.2-fold when the outcomes of the nab-paclitaxel treatment was compared with that of Cremophor-paclitaxel (Desai et al., 2006). Albumin is also thought to play a role in preferential intratumoral accumulation of paclitaxel. An in vivo study showed that, when the same doses of paclitaxel were administered, the intratumor paclitaxel accumulation was 33% higher for nab-paclitaxel compared with Cremophor EL-paclitaxel. It was suggested that this process might have been facilitated through binding of albumin to SPARC (secreted protein acid and rich in cysteine) (Desai et al., 2004). The SPARC is an extracellular matrix glycoprotein that is overexpressed and associated with poor prognosis in a variety of cancers, including breast cancer (Porter et al., 1995).

To understand the mechanism of the albumin-bound paclitaxel nanoparticles, the interfacial behavior of proteins at a water/oil interface should be taken into consideration. Previously, it was reported that proteins became denatured and/or aggregated at the water-in-oil (w/o) interface (Morlock et al., 1997). To compare the interfacial behavior of model proteins (albumin, lysozyme, or ovalbumin), 3 mL of aqueous protein solutions were emulsified in 12 mL of methylene chloride by use of a rotor/stator-type VirtiShear homogenizer (Sah, 1999). The compositions of water soluble protein species and water insoluble aggregates were analyzed before and after emulsification. Among 3 proteins tested, albumin showed the highest recovery of 98.7%, displaying its greatest stability. At the same time, it was found that water soluble dimeric and oligomeric species of albumin increased after emulsification. It was proven that reactions involving -SH and/or -S-S- were responsible for such changes. In other words, emulsification triggered thiol-thiol and/or thiol-disulfide bond reshuffling reactions. As a result, the amount of covalently linked, water

soluble albumin aggregates increased after emulsification . The importance of this interfacial behavior of albumin will be emphasized again in the following section.

It should also be mentioned that albumin, when physically associated with a poorly water-soluble drug such as paclitaxel, makes the drug more readily suspendable in an aqueous medium. Albumin also acts as a stabilizer to keep the nanoparticles from aggregation and/or congregation.

Preparation of albumin-bound paclitaxel nanoparticles

Information relevant to the detailed manufacturing process of albumin-coated paclitaxel nanoparticles are scarce. A series of patents issued to Dr. N. Desai disclose various methods of preparing albumin-bound paclitaxel nanoparticles. Some representative manufacturing processes are summarized in the below.

Preparation of albumin shells containing dissolved paclitaxel

Paclitaxel was dissolved in soybean oil to make a 2 mg/mL concentration. A 5% human serum albumin solution was overlayered with the soybean oil containing paclitaxel. The tip of the sonicator probe was positioned at the interface between the two solutions, and the sonicator turned on for 30 sec. In contrast to conventional methods for nanoparticle preparation, a surfactant such as SDS and polysorbate was not added to the mixture. Vigorous mixing occurred and a stable white milky suspension was obtained–Crosslinked albumin shells enclosed the oil/paclitaxel solution. In fact, the crosslinked albumin served as an emulsion stabilizer. A cosolvent (e.g., ethyl acetate having a higher taxol solubility than does soybean oil) can be added to soybean oil, in order to increase paclitaxel payload in the oil phase. After preparation of albumin shells containing dissolved paclitaxel, the organic solvent may be removed by evaporation under vacuum.

Preparation of crosslinked albumin associated with a solid paclitaxel core

Albumin-coated drug particle can be prepared as follows: An organic solvent (e.g., hexane, or ethyl ether, chloroform, methylene chloride, ethyl acetate, ethanol, tetrahydrofuran, dioxane, acetonitrile, acetone, dimethyl sulfoxide, dimethyl formamide, or methyl pyrrolidinone) can be used to dissolve paclitaxel at a relatively high concentration. The same procedure described earlier was followed. Five milliliters of the milky suspension of albumin shells containing dissolved paclitaxel were diluted in 0.9% NaCl solution to make a final volume of 10 mL. This suspension was placed in a rotary evaporator, and the volatile organic was removed by vacuum for 2 hr at room temperature. A microscope revealed opaque cores, indicating removal of substantially all organic solvent and the presence of solid paclitaxel within the crosslinked albumin shell. Alternatively, freeze-drying can be applied to remove the organic solvent.

The following is a little more detailed example of how to prepare nanoparticles by use of a high pressure homogenizer (Desai et al., 1999): 30 mg of paclitaxel were dissolved in 3 ml of methylene chloride. The organic phase was added to 27 mL of 1% human serum albumin solution. The mixture was emulsified for 5 min at a low rpm by a Vitris homogenizer, in order to make a crude emulsion. The emulsion was again emulsified by a high pressure homogenizer (Avestin) at 9,000-18,000 psi. At least 5 cycles of recycling were performed. The dispersion system was subject to rotary evaporation, to remove methylene chloride at 40ºC for 20-30 min. This treatment provided nanosized particles, and they were further lyophilized for 48 hr in the absence of any cryoprotectant. The resulting cake was well dispersed again by water or saline. The particle size remained unchanged before and after lyophilization. Since the particle size is less than 200 nm, the dispersion can be filtered through a 0.22 μm filter to prepare sterile nanoparticle injectables. Another interesting feature that this patent emphasizes is that the replacement of albumin with a typical surfactant leads to formation of large paclitaxel crystals, not paclitaxel nanoparticles. For example, 30 mg of paclitaxel was dissolved in 0.55 mL of chloroform and 0.05 mL of ethanol, and the resulting solution was added to 29.4 mL of 1% Tween 80 solution presaturated with 1% chloroform. When the same procedure describe earlier was carried out, large needle-like paclitaxel crystals with sizes of 0.7~5 μm were produced. When kept at room temperature, their crystal size increased to become precipitates.

In the above invention of nanoparticle preparation, no emulsifier is added to the mixture of two immiscible liquids. The reason is that the intermolecularly linked albumin molecules adsorb on the w/o interface, thereby stabilizing fine droplets. To induce intermolecular disulfide formation and/or thiol-disulfide bond reshuffling reactions, a device should provide high pressure, shear force, and cavitation. That is why a high pressure homogenizer or a sonicator is used for emulsification. It is contemplated that disulfide formation does not substantially denature albumin. The disulfide bond is also easily reduced under a reducing condition. In the practice of this manufacturing process, variables including protein concentration, temperature, sonication time and intensity, and drug concentration affect the physicochemical nature of the crosslinked albumin shells.

Differences between nab-paclitaxel and typical albumin nano/microparticles

There are several major differences between the nab^{TM}-paclitaxel and conventional albumin microspheres. In the practice of the nab^{TM} technology, albumin is crosslinked as mentioned above (Desai et al., 1999). High shear forces, cavitation, and local heating generate superoxide ions that oxidize the free sulfhydryl residues and/or disrupting existing disulfide bonds. As a consequence, a crosslinked albumin shell forms around nanodroplets of a dispersed phase containing paclitaxel. A transmission electron microscope demonstrated that the albumin shell thickness of the nab^{TM}-paclitaxel was by far thinner than the diameter of paclitaxel nanoparticles (Fig. 1C). By sharp contrast, conventional albumin nano/microspheres do not have albumin shells or coating: their entire matrices consist of albumin (Fig. 1A).

Paclitaxel raw powders are needle shaped crystals with sizes ranging from 5 to 500 μm. The study of X-Ray powder diffraction shows that paclitaxel in the albumin-coated nanoparticles is present as a substantially amorphous form, rather than a crystalline form. Conventional nano/microparticle preparation methods tend to lead to the formation of a crystalline form of paclitaxel (Liversidge et al., 1992; Merisko-Liversidge et al., 1996).

Clinical aspects of albumin-bound paclitaxel nanoparticles

A phase III study in patients with metastatic breast cancer demonstrated that the nab^{TM}-paclitaxel was favorable over the Cremophor-paclitaxel in terms of safety and efficacy (Gradishar et al., 2005). Patients (n = 229) were subject to 3-week cycles of intravenous administration of the nab^{TM}-paclitaxel 260 mg/m^2 over 30 min without premedication. On the other hand, patients (n = 225) received intravenous administration of Cremophor EL-paclitaxel 175 mg/m^2 over 3 hr with premedication. Even though the nab^{TM}-paclitaxel treatment group did not receive premedication, no severe hypersensitivity occurred to them. In comparison to the Cremophor EL-paclitaxel treatment the nab^{TM}-paclitaxel treatment accompanied not only increased response rates (33% versus 19%, respectively) and but also prolonging time to tumor progression (23.0 versus 16.9 weeks, respectively). As mentioned earlier, the albumin nanoparticle dosage form seems to contribute to improving the transcytosis of paclitaxel via an albumin receptor-mediated endothelial transport.

Future opportunity of nab^{TM} technology

The albumin-bound particle technology is being applied for other drugs such as docetaxel and rapamycin (Desai et al., 2010). The nab^{TM}-docetaxel also is a solvent-free, nanometer-sized form of docetaxel that targets various solid tumors. Since the nab^{TM} formulation does not require polysorbate 80 that is used in a conventional docetaxel formulation, the surfactant-associated toxicities might be eliminated. At the same time, the nab^{TM}-docetaxel is expected to increase the drug efficacy via its preferential albumin-driven deposition in tumors. Rapamycin, a protein kinase inhibitor, is under investigation as a cancer treatment. The poor water solubility and instability of rapamycin have limited its application as an anticancer agent. The nab^{TM}-rapamycin is expected to improve its aqueous solubility, thereby allowing intravenous administration. Even though albumin is presently used as the preferred nanocarrier protein, other proteins (e.g., lipoproteins, apolipoprotein B, α-acid glycoprotein, β_2-macroglobulin, thyroglobulin, transferin, fibronectin, and the like) might also have some potential. For instance, Chanasattru et al. prepared nanoparticles by heat treatment of β-lactoglobulin-pectin complexes (Chanasattru et al., 2009). Evaluating their feasibility as carrier proteins might deserve further study.

Conclusion

Albumin is an excellent nanocarrier protein, and parenteral formulations based on albumin particulate technology are growing strongly. In addition to ability to provide sustained drug release, albumin-based parenteral formulations allow targeted delivery of anticancer drugs to tumors, improve the bioavailability of poorly water soluble drugs, and avoid the use of hydrophobic solvents and surfactants.

Owing to these benefits, albumin particulate formulations would contribute to solving several problems associated with conventional solvent-based formulations. It is expected that albumin-based particulate technologies would extend their applications in delivering versatile bioactive materials including drugs, polypeptides, proteins, vaccines, nucleic acids, and genes.

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