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[54] **METHODS FOR IN VIVO DELIVERY OF SUBSTANTIALLY WATER INSOLUBLE PHARMACOLOGICALLY ACTIVE AGENTS AND COMPOSITIONS USEFUL THEREFOR**

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[58] **Field of Search** 424/451, 465, 450, 439; 260/403

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[57] **ABSTRACT**

In accordance with the present invention, there are provided compositions for the in vivo delivery of substantially water insoluble pharmacologically active agents (such as the anticancer drug taxol) in which the pharmacologically active agent is delivered in a soluble form or in the form of suspended particles. In particular, the soluble form may comprise a solution of pharmacologically active agent in a biocompatible dispersing agent contained within a protein walled shell. Alternatively, the protein walled shell may contain particles of taxol. In another aspect, the suspended form comprises particles of pharmacologically active agent in a biocompatible aqueous liquid.

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METHODS FOR IN VIVO DELIVERY OF SUBSTANTIALLY WATER INSOLUBLE PHARMACOLOGICALLY ACTIVE AGENTS AND COMPOSITIONS USEFUL THEREFOR

The present invention relates to in vivo delivery of substantially water insoluble pharmacologically active agents (e.g., the anticancer drug taxol). In one aspect, the agent is dispersed as a suspension suitable for administration to a subject, or is dissolved in a suitable biocompatible liquid. In another aspect, water insoluble pharmacologically active agents (e.g., taxol) are encased in a polymeric shell formulated from a biocompatible polymer. The polymeric shell contains particles of pharmacologically active agent, and optionally a biocompatible dispersing agent in which pharmacologically active agent can be either dissolved or suspended.

BACKGROUND OF THE INVENTION

Taxol is a natural product first isolated from the Pacific Yew tree, *Taxus brevifolia*, by Wani et al. [J. Am. Chem. Soc. Vol. 93:2325 (1971)]. Among the antimitotic agents, taxol, which contains a diterpene carbon skeleton, exhibits a unique mode of action on microtubule proteins responsible for the formation of the mitotic spindle. In contrast with other antimitotic agents such as vinblastine or colchicine, which prevent the assembly of tubulin, taxol is the only plant product known to inhibit the depolymerization process of tubulin, thus preventing the cell replication process.

Taxol, a naturally occurring diterpenoid, has been shown to have significant antineoplastic and anticancer effects in drug-refractory ovarian cancer. Taxol has shown excellent antitumor activity in a wide variety of tumor models such as the B16 melanoma, L1210 leukemias, MX-1 mammary tumors, and CS-1 colon tumor xenografts. Several recent press releases have termed taxol as the new anticancer wonder-drug. Indeed, taxol has recently been approved by the Federal Drug Administration for treatment of ovarian cancer. The poor aqueous solubility of taxol, however, presents a problem for human administration. Indeed, the delivery of drugs that are inherently insoluble or poorly soluble in an aqueous medium can be seriously impaired if oral delivery is not effective. Accordingly, currently used taxol formulations require a cremaphore to solubilize the drug. The human clinical dose range is 200–500 mg. This dose is dissolved in a 1:1 solution of ethanol:cremaphore and diluted to one liter of fluid given intravenously. The cremaphore currently used is polyethoxylated castor oil.

In phase I clinical trials, taxol itself did not show excessive toxic effects, but severe allergic reactions were caused by the emulsifiers employed to solubilize the drug. The current regimen of administration involves treatment of the patient with antihistamines and steroids prior to injection of the drug to reduce the allergic side effects of the cremaphore.

In an effort to improve the water solubility of taxol, several investigators have modified its chemical structure with functional groups that impart enhanced water-solubility. Among them are the sulfonated derivatives [Kingston et al., U.S. Pat. No. 5,059,699 (1991)], and amino acid esters [Mathew et al., J. Med. Chem. Vol. 35:145–151 (1992)] which show significant biological activity. Modifications to produce a water-soluble derivative facilitate the intravenous delivery of taxol dissolved in an innocuous carrier such as normal saline. Such modifications, however, add to the cost of drug preparation, may induce undesired side-reactions and-/or allergic reactions, and/or may decrease the efficiency of the drug.

Microparticles and foreign bodies present in the blood are generally cleared from the circulation by the 'blood filtering organs', namely the spleen, lungs and liver. The particulate matter contained in normal whole blood comprises red blood cells (typically 8 microns in diameter), white blood cells (typically 6–8 microns in diameter), and platelets (typically 1–3 microns in diameter). The microcirculation in most organs and tissues allows the free passage of these blood cells. When microthrombii (blood clots) of size greater than 10–15 microns are present in circulation, a risk of infarction or blockage of the capillaries results, leading to ischemia or oxygen deprivation and possible tissue death. Injection into the circulation of particles greater than 10–15 microns in diameter, therefore, must be avoided. A suspension of particles less than 7–8 microns, is however, relatively safe and has been used for the delivery of pharmacologically active agents in the form of liposomes and emulsions, nutritional agents, and contrast media for imaging applications.

The size of particles and their mode of delivery determines their biological behavior. Strand et al. [in *Microspheres-Biomedical Applications*, ed. A. Rembaum, pp 193–227, CRC Press (1988)] have described the fate of particles to be dependent on their size. Particles in the size range of a few nanometers (nm) to 100 nm enter the lymphatic capillaries following interstitial injection, and phagocytosis may occur within the lymph nodes. After intravenous/intraarterial injection, particles less than about 2 microns will be rapidly cleared from the blood stream by the reticuloendothelial system (RES), also known as the mononuclear phagocyte system (MPS). Particles larger than about 7 microns will, after intravenous injection, be trapped in the lung capillaries. After intraarterial injection, particles are trapped in the first capillary bed reached. Inhaled particles are trapped by the alveolar macrophages.

Pharmaceuticals that are water-insoluble or poorly water-soluble and sensitive to acid environments in the stomach cannot be conventionally administered (e.g., by intravenous injection or oral administration). The parenteral administration of such pharmaceuticals has been achieved by emulsification of the oil solubilized drug with an aqueous liquid (such as normal saline) in the presence of surfactants or emulsion stabilizers to produce stable microemulsions. These emulsions may be injected intravenously, provided the components of the emulsion are pharmacologically inert. U.S. Pat. No. 4,073,943 describes the administration of water-insoluble pharmacologically active agents dissolved in oils and emulsified with water in the presence of surfactants such as egg phosphatides, pluronics (copolymers of polypropylene glycol and polyethylene glycol), polyglycerol oleate, etc. PCT International Publication No. WO85/00011 describes pharmaceutical microdroplets of an anaesthetic coated with a phospholipid such as dimyristoyl phosphatidylcholine having suitable dimensions for intradermal or intravenous injection.

Protein microspheres have been reported in the literature as carriers of pharmacological or diagnostic agents. Microspheres of albumin have been prepared by either heat denaturation or chemical crosslinking. Heat denatured microspheres are produced from an emulsi-

fied mixture (e.g., albumin, the agent to be incorporated, and a suitable oil) at temperatures between 100° C. and 150° C. The microspheres are then washed with a suitable solvent and stored. Leucuta et al. [International Journal of Pharmaceutics Vol. 41:213–217 (1988)] describe the method of preparation of heat denatured microspheres.

The procedure for preparing chemically crosslinked microspheres involves treating the emulsion with glutaraldehyde to crosslink the protein, followed by washing and storage. Lee et al. [Science Vol. 213:233–235 (1981)] and U.S. Pat. No. 4,671,954 teach this method of preparation.

The above techniques for the preparation of protein microspheres as carriers of pharmacologically active agents, although suitable for the delivery of water-soluble agents, are incapable of entrapping water-insoluble ones. This limitation is inherent in the technique of preparation which relies on crosslinking or heat denaturation of the protein component in the aqueous phase of a water-in-oil emulsion. Any aqueous-soluble agent dissolved in the protein-containing aqueous phase may be entrapped within the resultant crosslinked or heat-denatured protein matrix, but a poorly aqueous-soluble or oil-soluble agent cannot be incorporated into a protein matrix formed by these techniques.

BRIEF DESCRIPTION OF THE INVENTION

Thus it is an object of this invention to deliver pharmacologically active agents (e.g., taxol, taxane, Taxotere, and the like) in unmodified form in a composition that does not cause allergic reactions due to the presence of added emulsifiers and solubilizing agents, as are currently employed in drug delivery.

It is a further object of the present invention to deliver pharmacologically active agents in a composition of microparticles suspended in a suitable biocompatible liquid.

It is yet another object of the invention to deliver pharmacologically active agents enclosed within a polymer shell which is further suspended in a biocompatible liquid.

These and other objects of the invention will become apparent upon review of the specification and claims.

In accordance with the present invention, we have discovered that substantially water insoluble pharmacologically active agents can be delivered in the form of microparticles that are suitable for parenteral administration in aqueous suspension. This mode of delivery obviates the necessity for administration of substantially water insoluble pharmacologically active agents (e.g., taxol) in an emulsion containing, for example, ethanol and polyethoxylated castor oil, diluted in normal saline (see, for example, Norton et al., in Abstracts of the 2nd National Cancer Institute Workshop on Taxol & Taxus, Sep. 23–24, 1992). A disadvantage of such known compositions is their propensity to produce allergic side effects.

The delivery of substantially water insoluble pharmacologically active agents in the form of a microparticulate suspension allows some degree of targeting to organs such as the liver, lungs, spleen, lymphatic circulation, and the like, through the use of particles of varying size, and through administration by different routes. The invention method of delivery further allows the administration of substantially water insoluble pharmacologically active agents employing a much smaller volume of liquid and requiring greatly reduced administration time relative to administration volumes and times required by prior art delivery systems (e.g., intravenous infusion of approximately one to two liters of fluid over a 24 hour period are required to deliver a typical human dose of 200–400 mg of taxol).

In accordance with another embodiment of the present invention, we have developed compositions useful for in vivo delivery of substantially water insoluble pharmacologically active agents. Invention compositions comprise substantially water insoluble pharmacologically active agents (as a solid or liquid) contained within a polymeric shell. The polymeric shell is a biocompatible polymer, crosslinked by the presence of disulfide bonds. The polymeric shell, containing substantially water insoluble pharmacologically active agents therein, is then suspended in a biocompatible aqueous liquid for administration.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided compositions for in vivo delivery of a substantially water insoluble pharmacologically active agent,

wherein said agent is a solid or liquid substantially completely contained within a polymeric shell,

wherein the largest cross-sectional dimension of said shell is no greater than about 10 microns,

wherein said polymeric shell comprises a biocompatible polymer which is substantially crosslinked by way of disulfide bonds, and

wherein said polymeric shell containing pharmacologically active agent therein is suspended in a biocompatible aqueous liquid.

As used herein, the term "in vivo delivery" refers to delivery of a pharmacologically active agent by such routes of administration as oral, intravenous, subcutaneous, intraperitoneal, intrathecal, intramuscular, inhalational, topical, transdermal, suppository (rectal), pessary (vaginal), and the like.

As used herein, the term "micron" refers to a unit of measure of one one-thousandth of a millimeter.

As used herein, the term "biocompatible" describes a substance that does not appreciably alter or affect in any adverse way, the biological system into which it is introduced.

Key differences between the pharmacologically active agents contained in a polymeric shell according to the invention and protein microspheres of the prior art are in the nature of formation and the final state of the protein after formation of the particle, and its ability to carry poorly aqueous-soluble or substantially aqueous-insoluble agents. In accordance with the present invention, the polymer (e.g., a protein) is selectively chemically crosslinked through the formation of disulfide bonds through, for example, the amino acid cysteine that occurs in the natural structure of a number of proteins. A sonication process is used to disperse a dispersing agent containing dissolved or suspended pharmacologically active agent into an aqueous solution of a biocompatible polymer bearing sulfhydryl or disulfide groups (e.g., albumin) whereby a shell of crosslinked polymer is formed around fine droplets of non-aqueous medium. The sonication process produces cavitation in the liquid that causes tremendous local heating and results in the formation of superoxide ions that crosslink the polymer by oxidizing the sulfhydryl residues (and/or disrupting existing disulfide bonds) to form new, crosslinking disulfide bonds.

In contrast to the invention process, the prior art method of glutaraldehyde crosslinking is nonspecific and essentially reactive with any nucleophilic group present in the protein structure (e.g., amines and hydroxyls). Heat denaturation as taught by the prior art significantly and irreversibly alters protein structure. In contrast, disulfide formation contemplated by the present invention does not substantially denature the protein. In addition, particles of substantially water insoluble pharmacologically active agents contained within a shell differ from crosslinked or heat denatured protein microspheres of the prior art because the polymeric shell produced by the invention process is relatively thin compared to the diameter of the coated particle. It has been determined (by transmission electron microscopy) that the "shell thickness" of the polymeric coat is approximately 25 nanometers for a coated particle having a diameter of 1 micron (1000 nanometers). In contrast, microspheres of the prior art do not have protein shells, but rather, have protein dispersed throughout the volume of the microsphere.

The polymeric shell containing solid or liquid cores of pharmacologically active agent allows for the delivery of high doses of the pharmacologically active agent in relatively small volumes. This minimizes patient discomfort at receiving large volumes of fluid and minimizes hospital stay. In addition, the walls of the polymeric shell are generally completely degradable in vivo by proteolytic enzymes (e.g., when the polymer is a protein), resulting in no side effects from the delivery system as is the case with current formulations.

According to this embodiment of the present invention, particles of substantially water insoluble pharmacologically active agents are contained within a shell having a cross-sectional diameter of no greater than about 10 microns. A cross-sectional diameter of less than 5 microns is more preferred, while a cross-sectional diameter of less than 1 micron is presently the most preferred for the intravenous route of administration.

Substantially water insoluble pharmacologically active agents contemplated for use in the practice of the present invention include pharmaceutically active agents, diagnostic agents, agents of nutritional value, and the like. Examples of pharmaceutically active agents include taxol (as used herein, the term "taxol" is intended to include taxol analogs and prodrugs, taxanes, and other taxol-like drugs, e.g., Taxotere, and the like), camptothecin and derivatives thereof (which compounds have great promise for the treatment of colon cancer), aspirin, ibuprofen, piroxicam, cimetidine, substantially water insoluble steroids (e.g., estrogen, prednisolone, cortisone, hydrocortisone, diflorasone, and the like), drugs such as phenesterine, duanorubicin, doxorubicin, mitotane, visadine, halonitrosoureas, anthrocylines, ellipticine, diazepam, and the like, anaesthetics such as methoxyfluorane, isofluorane, enfluorane, halothane, benzocaine, dantrolene, barbiturates, and the like. In addition, also contemplated are substantially water insoluble immunosuppressive agents, such as, for example, cyclosporines, azathioprine, FK506, prednisone, and the like. A presently preferred pharmaceutically active agent for use in the practice of the present invention is taxol, which is commercially available from the manufacturer as needle-like crystals.

Examples of diagnostic agents contemplated for use in the practice of the present invention include ultrasound contrast agents, radiocontrast agents (e.g., iodooctanes, halocarbons, renografin, and the like), magnetic contrast agents (e.g., fluorocarbons, lipid soluble paramagnetic compounds, and the like), as well as other diagnostic agents which cannot readily be delivered without some physical and/or chemical modification to accomodate the substantially water insoluble nature thereof.

Examples of agents of nutritional value contemplated for use in the practice of the present invention include amino acids, sugars, proteins, carbohydrates, fat-soluble vitamins (e.g., vitamins A, D, E, K, and the like) or fat, or combinations of any two or more thereof.

A number of biocompatible polymers may be employed in the practice of the present invention for the formation of the polymeric shell which surrounds the substantially water insoluble pharmacologically active agents. Essentially any polymer, natural or synthetic, bearing sulfhydryl groups or disulfide bonds within its structure may be utilized for the preparation of a disulfide crosslinked shell about particles of substantially water insoluble pharmacologically active agents. The sulfhydryl groups or disulfide linkages may be preexisting within the polymer structure or they may be introduced by a suitable chemical modification. For example, natural polymers such as proteins, oligopeptides, polynucleic acids, polysaccharides (e.g., starch, cellulose, dextrans, alginates, chitosan, pectin, hyaluronic acid, and the like), and so on, are candidates for such modification.

As examples of suitable biocompatible polymers, naturally occurring or synthetic proteins may be employed, so long as such proteins have sufficient cysteine residues within their amino acid sequences so that crosslinking (through disulfide bond formation, for example, as a result of oxidation during sonication) can occur. Examples of suitable proteins include albumin (which contains 35 cysteine residues), insulin (which contains 6 cysteines), hemoglobin (which contains 6 cysteine residues per $\alpha_2\beta_2$ unit), lysozyme (which contains 8 cysteine residues), immunoglobulins, α-2-macroglobulin, fibronectin, vitronectin, fibrinogen, and the like.

A presently preferred protein for use in the formation of a polymeric shell is albumin. Optionally, proteins such as α-2-macroglobulin, a known opsonin, could be used to enhance uptake of the shell encased particles of substantially water insoluble pharmacologically active agents by macrophage-like cells, or to enhance the uptake of the shell encased particles into the liver and spleen.

Similarly, synthetic polypeptides containing cysteine residues are also good candidates for formation of a shell about the substantially water insoluble pharmacologically active agents. In addition, polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrolidinone, and the like, are good candidates for chemical modification (to introduce sulfhydryl and/or disulfide linkages) and shell formation (by causing the crosslinking thereof).

In the preparation of invention compositions, one can optionally employ a dispersing agent to suspend or dissolve the substantially water insoluble pharmacologically active agent. Dispersing agents contemplated for use in the practice of the present invention include any nonaqueous liquid that is capable of suspending or dissolving the pharmacologically active agent, but does not chemically react with either the polymer employed to produce the shell, or the pharmacologically active

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