Albumin as a drug carrier: Design of prodrugs, drug conjugates and nanoparticles

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ABSTRACT

Albumin is playing an increasing role as a drug carrier in the clinical setting. Principally, three drug delivery technologies can be distinguished: coupling of low-molecular weight drugs to exogenous or endogenous albumin, conjugation with bioactive proteins and encapsulation of drugs into albumin nanoparticles. The accumulation of albumin in solid tumors forms the rationale for developing albumin-based drug delivery systems for tumor targeting. Clinically, a methotrexate-albumin conjugate, an albumin-binding prodrug of doxorubicin, i.e. the (6-maleimido)caproylhydrazone derivative of doxorubicin (DOXO-EMCH), and an albumin paclitaxel nanoparticle (Abraxane) have been evaluated clinically. Abraxane has been approved for treating metastatic breast cancer.

An alternative strategy is to bind a therapeutic peptide or protein covalently or physically to albumin to enhance its stability and half-life. This approach has been applied to peptides with antinociceptive, antidepressant, antitumor or antiviral activity: Levemir, a myristic acid derivative of insulin that binds to the fatty acid binding sites of circulating albumin, has been approved for the treatment of diabetes. Furthermore, Albuferon, a fusion protein of albumin and interferon, is currently being assessed in phase III clinical trials for the treatment of hepatitis C and could become an alternative to pegylated interferon. This review gives an account of the different drug delivery systems which make use of albumin as a drug carrier with a focus on those systems that have reached an advanced stage of preclinical evaluation or that have entered clinical trials.

1. Introduction

Albumin is emerging as a versatile protein carrier for drug targeting and for improving the pharmacokinetic profile of peptide- or protein-based drugs. Albumin is the most abundant plasma protein (35–50 g/L human serum) with a molecular weight of 66.5 kDa. Like most of the plasma proteins, albumin is synthesized in the liver where it is produced at a rate of approximately 0.7 mg/h for every gram of liver (i.e. 10–15 g daily); Human serum albumin (HSA) exhibits an average half-life of 19 days. The functions and binding properties of HSA are multifold [1]: a) it acts as the solubilizing agent for long chain fatty acids and is therefore essential for the metabolism of lipids; b) it binds bilirubin, the breakdown product of heme; c) it binds a great number of therapeutic drugs such as penicillins, sulfonamides, indole compounds, and benzodiazepines to name just a few; d) it binds copper(II) and nickel(II) in a specific and calcium(II) and zinc(II) in a relatively nonspecific manner and acts as the transport vehicle for these metal ions in the blood; e) it is the major protein responsible for the colloidal osmotic pressure of the blood; f) when HSA is broken down, the amino acids provide nutrition to peripheral tissue.

The three-dimensional structure of HSA has been elucidated by X-ray structure analysis [2,3]. The approximate three-dimensional shape of HSA can be described as an ellipsoid consisting of three flexible spheres in a row (domains I, II, III) and is illustrated schematically in Fig. 1. HSA is one of the smallest proteins present in blood plasma. Both size and abundance explain the fact that so many metabolic compounds and therapeutic drugs are transported by this protein. The binding sites for metabolic substrates and diagnostic as well as therapeutic drugs have been extensively studied and reviewed [4,5].

Albumin is used for treating shock, burns, hypoalbuminemia, surgery or trauma, cardiopulmonary bypass, acute respiratory distress and hemodialysis [6]. An alternative to blood derived albumin, recombinant human serum albumin (Recombumin) has been developed and is a genetically engineered protein expressed in yeast cells that has shown comparable safety, tolerability, pharmacokinetics and pharmacodynamics to native HSA [7].

Albumin is an acidic, very soluble protein that is extremely robust: it is stable in the pH range of 4–9, soluble in 40% ethanol, and can be heated at 60 °C for up to 10 h without deleterious effects. These properties as well as its preferential uptake in tumor and inflamed tissue, its ready availability, its biodegradability, and its lack of toxicity and immunogenicity make it an ideal candidate for drug delivery.
2. Albumin as a drug carrier

Albumin accumulates in malignant and inflamed tissue due to a leaky capillary combined with an absent or defective lymphatic drainage system. Tumor uptake in preclinical models can be easily visualized by injecting the dye Evans blue that binds rapidly and tightly to circulating albumin and makes subcutaneously growing tumors turn blue within a few hours post-injection (see Fig. 2). As an alternative to drug targeting, conjugating therapeutic peptides or cytokines with albumin is an attractive approach of improving their pharmacokinetic profile due to the long-half-life of albumin in the body. The rationale for the different drug delivery strategies is outlined below.

2.1. Accumulation of albumin in solid tumors

In the middle of the 20th century the first reports appeared in the literature demonstrating that tumors are able to trap plasma proteins and utilize their degradation products for proliferation [8]. An important study concerning the uptake of plasma proteins of varying size by mouse tumors was reported in 1986 [9]. One of the results of this study was that the pharmacokinetic profile of the different proteins studied (molecular weights ranging from 12 to 150 kDa) correlated with the rate of tumor uptake: a long half-life in the blood circulation proved to be a prerequisite for an enhanced tumor uptake of the protein. Furthermore, there was no significant difference in tumor accumulation between albumin (MW 66.5 kDa) and an immunoglobulin (MW 150 kDa). These and other studies concerning tumor uptake, tumor blood flow and the transport of molecules in the interstitium led Maeda and Matsumura to coin the expression “EPR”, i.e. enhanced permeability and retention of macromolecules in relation to passive tumor targeting [10]. The leaky defective blood vessels of tumor tissue make its vasculature permeable for macromolecules whereas in blood vessels of healthy tissue only small molecules can pass the endothelial barrier. The pore size of tumor microvessels varies from 100 to 1200 nm in diameter [11,12]. Macromolecules employed as carriers for the development of macromolecular produgs typically have hydrodynamic radii that are >2 nm and <10 nm (e.g., serum albumin has an effective diameter of 7.2 nm) allowing extravasation into tumor tissue but not into normal tissue.

The enhanced uptake of macromolecules in tumor tissue cannot be solely explained by an enhanced permeability of the vascular system since this would affect smaller molecules in a similar manner, but is also due to a reduced clearance from the tumor when the molecular weight exceeds 40 kDa [13]. Whereas smaller molecules were shown to be rapidly cleared from the tumor interstitium, large molecules are retained thus showing high intratumor concentrations even after 100 h post application [13]. This enhanced retention of macromolecules in tumor tissue is primarily caused by a lack of lymphatic drainage due to an impaired or absent lymphatic system. Hence, it is the combination of both an enhanced permeability and retention (EPR) that is responsible for the accumulation of macromolecules in solid tumors.

From 1990 onwards an increasing number of distribution studies concerning the uptake of labeled albumin in animal tumors appeared in the literature. These studies, in which albumin was either radiolabeled or conjugated with dyes, showed that between 3% and 25% of the applied dose was found in the tumor (reviewed in [14]).

As an example, scintigraphic images of rats bearing ovarian tumors of different size or a Walker-256 carcinoma in the left hind leg 72 h after administration of [111In]-DTPA labeled rat serum albumin are shown in Fig. 2. As can be seen, the amounts of tracer substance increase with tumor weight and more than 20% of a single dose of radiolabeled albumin accumulates in large tumors. As an explanation for the high albumin turnover in rodent tumors, Stehle et al. have proposed that albumin is a major energy and nutrition source for tumor growth [15]. Their underlying hypothesis on tumor nutrition is based on an excessive plasma protein catabolism by the tumor itself and an active metabolic role of the liver which seem to be important factors for the genesis of cachexia.
2.2. Accumulation of albumin in inflamed joints in arthritic disease

Similar to cancer patients with cachexia [15], patients with active rheumatoid arthritis frequently develop hypoaalbuminemia that is primarily caused by high albumin consumption at sites of inflammation [16–18]. The metabolism of synovial cells is highly up-regulated, and uptake of albumin is probably a relevant source of covering their high demand for nitrogen and energy. The permeability of the blood-joint barrier for albumin in inflamed joints of rheumatoid arthritis patients is markedly increased [19]. In preclinical models, Wunder and co-workers have nicely shown that albumin accumulates in the arthritic paws of mice suffering from collagen-induced arthritis [20]. Using scintigraphy and laseroptical methods with fluorescein-labeled or radiolabeled albumin, they were able to demonstrate an intensive accumulation of albumin in paws affected by arthritis as shown in Fig. 3 [21]. Albumin is thus an attractive drug carrier to target drugs to inflamed joints of patients with rheumatoid arthritis and the antirheumatic drug methotrexate bound to albumin has shown promising activity in the collagen-induced arthritis model as outlined in Section 3.2.

2.3. Improving the pharmacokinetic properties of therapeutically active peptides and proteins

Therapeutically relevant peptides and proteins are playing an increasing role in the treatment of viral, malignant and autoimmune diseases. The development and successful application of therapeutic peptides or proteins, however, is often impeded by several difficulties which includes insufficient stability and shelf-life, costly production, immunogenic and allergic potential, as well as poor bioavailability and sensitivity towards peptidases. Efforts to reduce peptide cleavage by altering peptides or inhibiting peptidases have had mixed success and have not been able to address rapid kidney clearance of small peptides by glomerular filtration.

Three elegant technologies to overcome most of these difficulties that are based on albumin as the drug carrier have been proposed: The Albumin Fusion Technology developed by Human Genome Sciences, Inc. [22], The Drug Affinity Complex (DAC™) and Preformed Conjugate-Drug Affinity Complex (PC-DAC™) technology platforms developed by ConjuChem, Inc. [23], and the development of peptide fatty acid derivatives that bind physically to circulating albumin developed by Novo Nordisk [24].

Albumin Fusion Technology yields an altered version of a protein by fusing the gene for human albumin to the gene that encodes the active protein drug. This technology has initially been applied to cytokines such as interferons and interleukins (see Section 3.4), and similar to pegylation of the native protein [25] increases its molecular weight and as a result prolongs the half-life in vivo which allows less frequent administration of the therapeutic protein. In addition, the albumin molecule masks the protein rendering it more resistant to proteases and less immunogenic.

To improve the pharmacokinetic profile of low-molecular weight peptides, which generally have a plasma half-life of only a few minutes, ConjuChem, Inc. have developed a technology in which therapeutically active peptides are bound to albumin and coined the expression Drug Affinity Complex (DAC) technology to describe their constructs. Each DAC construct consists of the drug, a connector molecule and a reactive chemistry group that is responsible for the permanent bonding of the construct to target proteins. Typical reactive chemistry groups are N-hydroxysuccinimide esters, isocyanates, and a maleimide or a salicylate group. For each DAC construct, the site of attachment of the connector to the drug as well as the length of the connector is optimized to ensure that the drug retains a substantial proportion of the activity of the parent drug in its protein-bound form. The majority of DAC peptides have been realized with a maleimide group that reacts rapidly and specifically with the cysteine-34 position of serum albumin. The cysteine-34 position of albumin that is located in subdomain IA of albumin is highly conserved in all mammalian species studied with the exception of salmon albumin [1,3]. The free HS-group of cysteine-34 is an unusual feature of an extracellular protein. The X-ray structure of defatted albumin reveals that cysteine-34 is located in a hydrophobic crevice on the surface of the protein that is approximately 10–12 Å deep (see Fig. 4 A, B). When HSA is complexed with long-chain fatty acids as in the X-ray structure in which five molecules of myristic acid are bound, the crevice is opened up exposing the HS-group of cysteine-34.

The Drug Affinity Complex (DAC™) and Preformed Conjugate-Drug Affinity Complex (PC-DAC™) technology platforms developed by ConjuChem, Inc. primarily target this unique amino acid on the surface of exogenous or endogenous albumin: The reaction of the maleimide functionalized peptide occurs either in vivo using endogenous albumin (for DAC™) or ex vivo using recombinant albumin (for PC-DAC™). Through binding to albumin, the DAC™ and PC-DAC™ peptides are protected from rapid degradation and excretion. In addition, these peptides effectively adopt the beneficial pharmacokinetic properties of albumin, i.e. they are distributed throughout nearly all tissues and organs in the body and do not easily cross the blood-brain barrier, thus preventing most central nervous system side effects. Clinically, the most advanced peptide drug is CJC-1134-PC, an albumin conjugate of Exendin-4, a glucagon-like peptide-1 (GLP-1) homolog for treating type 2 diabetes (see Section 3.4).
I summarize most of the pertinent developments in the field of drug albumin conjugates and albumin-binding drug derivatives.

3. Drug albumin conjugates and albumin-binding drug derivatives

Historically, the first drug albumin conjugates were synthesized by direct coupling methods followed by the development of albumin-binding peptides and prodrugs that bind rapidly and selectively to the cysteine-34 position of exogenous and endogenous albumin [23,26]. In addition, drug albumin conjugates that contain an appropriate ligand for receptor targeting such as sugars [27] or RGD peptides [28] for application in liver and vascular targeting have been developed. In the diagnostic field, fluorescein-labeled albumin and $^{99m}$Tc-galactosyl human serum albumin (TcGSA) could find application in laser-induced fluorescence imaging for delineating tumor margins under the operating microscope or for diagnosing liver disease [29,30]. Below, I summarize most of the pertinent developments in the field of drug albumin conjugates and albumin-binding drug derivatives.

3.1. Albumin conjugates and albumin-binding prodrugs with anticancer agents

The first drug albumin conjugate that was evaluated in phases I/II clinical studies was a methotrexate-albumin conjugate (MTX-HSA). MTX-HSA was synthesised by directly coupling the drug to lysine residues of HSA. It was found that the drug-loading ratio significantly determined the tumor targeting properties of MTX-albumin conjugates in rats. In a systematic study, in which the tumor uptake of MTX-HSA loaded with 1, 5, 7, 10, or 20 molecules of the drug was compared in Walker-256 carcinoma bearing rats, only loading rates of close to one equivalent MTX per molecule of albumin offered optimal tumor targeting properties comparable to unmodified albumin [31,32]. The MTX-HSA conjugate loaded with 1.3 equivalents of MTX has shown promising antitumor efficacy in various animal models. A clinical phase I study has been performed with 17 patients treated with weekly MTX-HSA [33]. Stomatitis proved to be dose-limiting above 50 mg/m$^2$ MTX-HSA (MTX equivalents). A noteworthy finding of this study was that two patients with renal cell carcinoma and one patient with mesothelioma responded to MTX-HSA therapy (one partial response, two minor responses). However, it was not possible to confirm these results in a subsequent phase II study in 17 patients with metastatic renal carcinoma in which no objective response was seen [34]. Another phase II study with MTX-HSA in combination with cisplatin was conducted for the first line treatment of patients with advanced bladder cancer [35]. Treatment was started with a loading dose of 110 mg/m$^2$ of MTX-HSA followed by a weekly dose of 40 mg/m$^2$. Cisplatin was given monthly at a dose of 75 mg/m$^2$. One complete and one partial remission were observed in seven evaluable patients. However, there is currently no indication that the clinical assessment of MTX-HSA is being further pursued.

Drawbacks of MTX-HSA are (1) that the conjugate is not chemically clearly defined because varying amounts of methotrexate are bound to albumin yielding an average of ~1.3 molecules per albumin molecule and (2) the prodrug nature of MTX-HSA remains unclear with respect to cleavage rate and cleavage products.

As a consequence, Kratz and co-workers focused on bioconjugation methods that would improve the coupling methods of drug derivatives and obtain better defined drug albumin conjugates having high purity, a constant drug-loading ratio, a minimal alteration of the three-dimensional protein structure as well as a predetermined breaking point. Commercially available albumin is a mixture of mercapitalbumin and nonmercapitalbumin containing approximately 20–60% free sulfhydryl groups per molecule albumin due to the fact that the cysteine-34 position is blocked by sulfhydryl compounds such as cysteine, homocysteine or glutathione. We therefore developed a procedure of selectively reducing HSA with suitable agents, such as dithiothreitol (Cleland’s reagent), in a first step so that approximately one sulfhydryl group per molecule albumin can be determined. In a second step, doxorubicin maleimide derivatives such as the 4-maleimidophenylacetyl hydrazone derivative of doxorubicin (abbreviated DOXO-HYD) were coupled to this reduced form of albumin [36]. The resulting conjugate A-DOXO-HYD was distinctly superior compared to free doxorubicin against murine renal carcinoma (RENCA) compared to free doxorubicin at equitoxic dose [36].

Encouraged by these results, we focused our work on a prodrug concept that exploits endogenous albumin as a drug carrier [26,37]. In this therapeutic strategy, the prodrug is designed to bind rapidly and selectively to the cysteine-34 position of circulating serum albumin after intravenous administration thereby generating a macromolecular transport form of the drug in situ in the blood. We reasoned that exploiting circulating albumin as a drug carrier would have several advantages over ex vivo synthesized drug albumin conjugates: (a) the
use of commercial and possibly pathogenic albumin is avoided; (b) albumin-binding drugs are chemically well-defined and based on straightforward organic chemistry; (c) albumin-binding drugs are fairly simple and inexpensive to manufacture; (d) a broad range of drugs for developing albumin-binding drugs can be used; (e) the analytical requirements for defining the pharmaceutical products are comparable to any other low-molecular weight drug candidate.

The macromolecular prodrug approach targets the cysteine-34 position of albumin. Approximately 70% of circulating albumin in the blood stream is mercaptoalbumin containing an accessible cysteine-34, which is not blocked by endogenous sulphydryl compounds. Considering that free thiol groups are not found on the majority of circulating serum proteins except for albumin, cysteine-34 of endogenous albumin is a unique amino acid on the surface of a circulating protein.

Proof of concept was obtained with a two-acid-sensitive doxorubicin prodrugs, i.e., the (4-maleimidophenylacetyl)hydrazone derivative of doxorubicin and the (6-maleimidocaproyl)hydrazone derivative of doxorubicin (DOXO-EMCH) that are rapidly and selectively bound to circulating albumin within a few minutes [26,37]. Therapy with DOXO-EMCH dramatically improved the efficacy of doxorubicin in preclinical tumor models [36]. As an example the antitumor efficacy of DOXO-EMCH was compared to that of doxorubicin in the MDA-MB 435 model at the following doses: doxorubicin: 2×8 mg/kg, DOXO-EMCH: 2×8 mg/kg, 3×16 mg/kg and 3×24 mg/kg doxorubicin equivalents. Preliminary toxicity studies in nude mice had shown that the maximum tolerated dose of DOXO-EMCH was approximately 4.5 times higher than for free doxorubicin. The results of this animal experiment are shown in Fig. 5A. At the MTD of free doxorubicin (2×8 mg/kg), a moderate inhibition in tumor growth is observed with DOXO-EMCH comparable to the effect of free doxorubicin at the same dose. At higher doses therapy with DOXO-EMCH produced good antitumor effects at 3×16 mg/kg doxorubicin equivalents and complete remissions at 3×24 mg/kg.

The biodistribution of 14C-labeled DOXO-EMCH and doxorubicin was assessed in this model, and the results after 2, 6, 24, and 48 hours are shown in Fig. 5B for serum, tumor, heart, liver and kidneys. As expected, there is a pronounced difference between the radioactivity levels of DOXO-EMCH and doxorubicin in the serum: whereas levels for serum doxorubicin are below 0.3% of the applied dose for all time points, levels for DOXO-EMCH after 2 hours are ~25% and still ~6% of the applied dose after 48 hours which is a clear indication of rapid binding of DOXO-EMCH to albumin and a large AUC in the blood pool. Tumor levels for DOXO-EMCH increase over 24 hours and are approximately 2-fold higher between 24 and 48 hours compared to free doxorubicin levels. In contrast, the levels in heart, liver and kidneys are significantly lower for DOXO-EMCH than for doxorubicin over several hours after intravenous administration.

DOXO-EMCH was selected as the investigational product for clinical evaluation after toxicology studies in mice, rats, and dogs had shown that DOXO-EMCH exhibits a 2- to 5-fold increase in the maximum tolerated dose (MTD) in these animals when compared to conventional doxorubicin and a better tolerability in a 4-cycle study in rats and a 2-cycle study in dogs even at a 3-fold higher dose for DOXO-EMCH than for doxorubicin [38]. DOXO-EMCH has also shown significantly less chronic cardiotoxicity at equimolar as well as equitoxic doses compared to doxorubicin in a rat model [39].

In summary, the major difference between doxorubicin and DOXO-EMCH is the substantial increase in the MTD. As a consequence, the therapeutic index of DOXO-EMCH is significantly enhanced allowing high doses to be administered to tumor-bearing animals with a concomitant increase in antitumor activity compared to free doxorubicin. In general, a high degree of protein-binding, especially to albumin, is considered a disadvantage because only the free drug can exert its pharmacological effect. In situ binding of prodrugs to albumin turns this potential disadvantage into a therapeutic benefit by incorporating a cleavable bond between the drug and the albumin-binding moiety that ensures a specific release of the drug at its site of action.

In a phase 1 study a starting dose of 20 mg/m² doxorubicin equivalents was chosen and 41 patients with advanced cancer disease were treated at dose levels of 20–340 mg/m² doxorubicin equivalents [40]. Treatment with DOXO-EMCH was well tolerated up to 200 mg/m² without manifestation of drug-related side effects which is a ~3-fold increase over the standard dose for doxorubicin (60 mg/kg). Myelosuppression and mucositis were the predominant adverse effects at dose levels of 260 mg/m² and became dose-limiting at 340 mg/m². Pharmacokinetically, the albumin-bound form of DOXO-EMCH has a large AUC, a small volume of distribution and low clearance compared to doxorubicin, and there are some clear similarities, but also differences to liposomal doxorubicin (Doxil) as can be noted when comparing the data presented in Table 1.

30 of 41 patients were assessable for analysis of response. Partial responses were observed in 3 patients (10%), small cell lung cancer, liposacoma and mamma carcinoma. 15 patients (50%) showed a stable disease at different dose levels and 12 patients (40%) had evidence of tumor progression. A phase II study against small cell lung cancer will be initiated in 2008 to assess the antitumor potential of DOXO-EMCH, renamed INNO-206 by Innovive Pharmaceuticals.

Inspired by the translational research with DOXO-EMCH, a broad spectrum of albumin-binding prodrugs has been developed by Kratz and co-workers (see Fig. 6). These prodrugs consist of an anticancer drug, the maleimide group as the thiol-binding moiety and an enzymatically cleavable peptide linker. Examples include doxorubicin prodrugs that are cleaved by matrix metalloproteases 2 and 9 [41], cathepsin B [42], urokinase [43] or prostate-specific antigen (PSA) [44,45], metotrexate prodrugs that are cleaved by cathepsin B and plasmin [46], and camptothecin prodrugs that are cleaved by cathepsin B or unidentified proteases [42,47,48]. In addition, maleimide derivatives with 5-fluorouracil analogues and platinum(ii) complexes have been developed [49,50].

An extension of the in situ albumin technology is the current development of novel albumin-binding prodrugs that combine passive and active targeting or act as dual acting prodrugs (see Fig. 7). In the first strategy, a receptor-recognizing ligand is additionally introduced in the prodrug constuct. Examples for suitable receptors are the folate receptor, integrins or the asialoglycoprotein receptor that are overexpressed by various solid tumors, the tumor endothelium and liver tumors, respectively. Through in situ binding of a ligand-based albumin-binding prodrug to endogenous or exogenous albumin a modified albumin drug conjugate is formed that besides passive uptake in solid tumors has the potential to preferentially interact with tumor-associated receptors or antigens and improve overall tumor targeting.

The second new approach relies on binding two drugs to albumin (see Fig. 7). In its simplest form these can two be two anticancer agents for a cellular combination therapy approach or the dual acting prodrug consists of a drug such as an anticancer agent and the second drug is a modulator, e.g. an inhibitor of the p-glycoprotein or an inducer of apoptosis. The goal of such prodrugs is to circumvent chemoresistance of solid tumors, a pivotal and unresolved issue in cancer chemotherapy.

3.2. Albumin conjugates and albumin-binding prodrugs with the antirheumatic drug methotrexate

Methotrexate (MTX) is the most common drug used in the treatment of rheumatoid arthritis. However, progression of joint destruction in most patients cannot be completely inhibited by MTX treatment. Due to increased extravasation of albumin in inflamed tissue and metabolism of albumin by cells of the rheumatoid synovial pannus, albumin is an attractive carrier for antirheumatic
drugs such as MTX (see Section 2.2). MTX-HSA, the identical methotrexate-albumin conjugate that has been assessed preclinically as well as clinically as an antitumor agent, was investigated for anti-inflammatory activity in mice suffering from collagen-induced arthritis. In comparison to equivalent concentrations of MTX, MTX-HSA was significantly more effective than MTX in the prevention of collagen-induced arthritis and acts synergistically to MTX in this model [51]. In a human model of rheumatoid arthritis using severe combined immunodeficient mice which were co-transplanted with human cartilage and synovial fluid from patients with rheumatoid arthritis, synovial fibroblast invasion and cartilage degradation was reduced by MTX-HSA in vivo [52].

In order to avoid the use of exogenous and possibly pathogenic albumin and to obtain a better defined pharmaceutical product, we developed an albumin-binding prodrug of methotrexate, EMC-D-Ala-Phe-Lys-Lys(γ-MTX)-OH (EMC = 6-maleimidocaproic acid), that rapidly binds to endogenous albumin to form a conjugate which is stable in human plasma (see Fig. 8). Due to two lysine residues it can be cleaved by cathepsin B and plasmin, two enzymes which are found in high levels in the synovial joints of patients with rheumatoid

Fig. 5. A. Curves depicting tumour growth inhibition of subcutaneously implanted MDA-MB-435 xenografts under therapy with doxorubicin and DOXO-EMCH; B. Biodistribution study in the MDA-MB-435 xenograft model with 14C-labeled doxorubicin or DOXO-EMCH (organ values were corrected for blood volume); p < 0.05.

DOXO-EMCH
arthritis [53]. EMC-D-Ala-Phe-Lys-Lys(γ-MTX)-OH, by binding endogenous albumin, has the potential to accumulate in inflamed joints where a MTX-lysine derivative of the drug is released either extra- or intracellularly. EMC-D-Ala-Phe-Lys-Lys(γ-MTX)-OH suppressed collagen-induced arthritis in a dose-dependent manner and was superior to MTX, especially at later stages of the disease. In an intermediate stage of collagen-induced arthritis, treatment was started at day 30 after induction of arthritis and monitored for 24 days. When the mice were randomized for treatment, all animals had active disease with a moderate arthritis score of 7.3 ± 3.5 SD. In this experiment the MTX prodrug showed a clear reduction of the arthritis score with a significant difference when compared to the saline control and the MTX group, the latter being without any effect (see Fig. 8). These results indicate that the albumin-binding MTX prodrug might be effective in treating progressed rheumatoid arthritis where MTX lacks efficacy.

3.3. Albumin conjugates for liver targeting

A further application of albumin in drug delivery is liver targeting using albumin conjugates containing galactose residues. These neoglycoprotein conjugates are designed to selectively enter hepatocytes by binding to the asialoglycoprotein receptor with subsequent internalization and degradation of the carrier in lysosomes, thus circumventing extrahepatic side effects such as neurotoxicity of antiviral nucleoside analogues in the treatment of chronic viral hepatitis. The validity of this therapeutic strategy has been demonstrated in a clinical study in which adenine arabinoside monophosphate (ara-AMP) conjugated to

![Fig. 6. Structures of a selection of albumin-binding prodrugs.](image)

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>$t_{1/2}$ terminal (h)</th>
<th>$C_{max}$ (μM)</th>
<th>AUC (μM h)</th>
<th>$V_z$ (L)</th>
<th>CL (mL/min)</th>
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<tr>
<td>Doxorubicin (60 mg/m²)</td>
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<td>-3.5</td>
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<td>-1000</td>
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<tr>
<td>DOXO-EMCH (80 mg/m²)</td>
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<td>-28</td>
<td>-520</td>
<td>-5.8</td>
<td>-9</td>
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<tr>
<td>DOXO-EMCH (260 mg/m²)</td>
<td>-20</td>
<td>-235</td>
<td>-2550</td>
<td>-4.6</td>
<td>-8.5</td>
</tr>
<tr>
<td>Doxil (60 mg/m²)</td>
<td>56–90</td>
<td>30–47</td>
<td>2340–4070</td>
<td>3.0–5.6</td>
<td>0.3–1.25</td>
</tr>
</tbody>
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Values taken from reference [101].

were randomized for treatment, all animals had active disease with a moderate arthritis score of 7.3 ± 3.5 SD. In this experiment the MTX prodrug showed a clear reduction of the arthritis score with a significant difference when compared to the saline control and the MTX group, the latter being without any effect (see Fig. 8). These results indicate that the albumin-binding MTX prodrug might be effective in treating progressed rheumatoid arthritis where MTX lacks efficacy.

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lactosaminated albumin exerted an antiviral effect comparable to the free drug without producing any major side effects including the severe neurotoxicity of free ara-AMP [54].

Fiume and co-workers adapted their carrier system for a potential treatment of hepatocellular carcinoma [55–57]. In a study on the needle biopsies of 60 human hepatocellular carcinoma, the asialoglycoprotein receptor was histochemically detected in 80% well differentiated and in 20% poorly differentiated forms of the tumor [58] which forms the basis for exploiting the asialoglycoprotein receptor as a molecular target for the selective delivery of drugs to hepatocellular carcinoma.

In line with this rationale, the (6-maleimidocaproyl)hydrazone derivative of doxorubicin (DOXO-EMCH) was coupled to a thiolated derivative of doxorubicin (DOXO-EMCH) was coupled to a thiolated human albumin (L-HSA). The resulting conjugate L-HSA-DOXO achieved a very efficient targeting of the drug to the liver of treated mice with doxorubicin concentrations reaching levels 7–20 times higher than those raised in extrahepatic tissues [55]. In further experiments against hepatocellular carcinoma induced in mice by NN-diethylnitrosamine, L-HSA-DOXO at a dose of 4×1 mg/kg doxorubicin equivalents significantly inhibited tumor growth without decreasing body weights [57]. In contrast, free doxorubicin administered at the same dose as the coupled drug, did not affect tumor growth and produced a significant decrease in the body weight of the treated animals. Experiments in healthy rats have shown that even a dose of 4×2 mg/kg L-HSA-DOXO that is twice the dose of that used in the therapeutic model produces essentially no liver toxicity indicating an excellent therapeutic index for the novel conjugate [56].

### 3.4. Albumin-binding derivatives with therapeutically active peptides

The Drug Affinity Complex (DAC) technology from ConjuChem, Inc., has been especially attractive for improving the efficacy and pharmacokinetic profile of thrombin inhibitors and therapeutic peptides with antidiabetic, antinociceptive, hypotensive or antiviral activity [59]. Earlier work focused on NHS-derivatives of thrombin and rennin inhibitors that were bound to albumin and retained activity in vitro [59]. In addition, peptide derivatives of dynorphin A, H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Leu-Lys-NH₂, which contain a maleimide group as the albumin-binding moiety have been developed. A major limitation of opioid therapy with dynorphins is central mediated side effects. By binding dynorphin A to albumin which is present in the central nervous system in low concentrations, it could be possible to avoid these side effects and also enhance the pain-relieving effect peripherally for an extended period. The maleimido-acid derivative of this peptide (CJC-1008) bound to the cysteine-34 position of albumin has shown in vitro antinoceptive activity and longer duration of action in vivo [60] and advanced to clinical trials. A phase II study provided preliminary evidence of a greater analgesic effect when using CJC-1008 compared to placebo in patients with postherpetic neuralgia; unfortunately, the effect only lasted for 8 h and diminished by 24 h. No further clinical trials with this albumin-binding derivative have been initiated.

Clinically, the most advanced DAC construct is CJC-1134-PC, an albumin conjugate of exendin-4, a glucagon-like peptide-1 (GLP-1) homolog for treating type 2 diabetes. GLP-1 analogues stimulate insulin synthesis in the pancreas and have shown a good safety profile as novel antidiabetic agents [61]. However, GLP-1 analogues have a very short serum half-life of ~5 min to 1 h due to cleavage by peptidases.

As a consequence, in their initial work ConjuChem, Inc. developed a maleimide derivative of GLP-1, CJC-1131, designed as an albumin-binding peptide derivative which binds rapidly and selectively to the cysteine-34 position of circulating albumin [62]. CJC-1131 was active in experimental diabetes models [62] and was generally well tolerated in rats and beagle dogs [23]. Interestingly, CJC-1131 could be administered intravenously as well as subcutaneously, and once bound to endogenous albumin demonstrated a half-life of 15 to 20 h in rats and ~9–15 days in humans after subcutaneous administration [23]. Four phase I/I trials have shown that CJC-1131 is well tolerated and no signs of immunogenicity were noted in multiple-dose regimens [23].

However, ConjuChem, Inc. decided to continue their clinical development with a different conjugate, i.e., CJC-1134-PC (PC-DAC™). Exendin-4, possibly due to formulation issues with CJC-1131 or owing to the fact that exendin-4 is more potent at lowering glucose concentrations than human GLP-1. Exendin-4 is a GLP-1 homolog and an agonist for the GLP-1 receptor that lowers the blood glucose by decreasing glucagon and increasing insulin secretion in a glucose-dependent manner. Exendin-4 may stimulate β-cell proliferation,
restore β-cell sensitivity to glucose, delay gastric emptying, and increase peripheral sensitivity to glucose. Two exendin-4 derivatives are being developed by Eli Lilly/Amylin Pharmaceuticals and Aventis. The clinical application of low-molecular weight exendin-4 derivatives, however, is limited by their relatively short half-life in plasma (t1/2 <1 h). QJC-1134-PC, the modified maleimide exendin-4 analogue conjugated to the cysteine-34 position of recombinant human albumin, is based on the PC-DAC™ technology. This preformed conjugate is a long-acting antidiabetic albumin conjugate that demonstrated a good tolerability profile and positive efficacy on glucose reduction in a phase I/II trial starting once-a-week dosing. The albumin conjugate is injected subcutaneously providing excellent compliance for diabetes patients (www.conjuchem.com).

ConjuChem have an albumin conjugate with insulin (PC-DAC™: Insulin) and an albumin-binding derivative of a C34 peptide that targets gp41 of the human immunodeficient virus (HIV) (DAC™:HIV) under preclinical development—see www.conjuchem.com.

An alternative to chemical conjugation of bioactive peptides to albumin is the development of peptide derivatives that bind physically to circulating albumin. This approach is exemplified by the development of MN304, a long-acting insulin analogue that is acylated with a C14-fatty acid chain [24]. It is formed by removal of threonine at position B30 of native insulin, and the addition of a C14 fatty acid at B29 and, as a consequence, is 98% reversibly bound to free fatty acid binding sites on albumin in plasma and interstitial fluids [63]. Albumin binding prolongs duration of action of insulin and contrasts with existing long-acting insulins whose duration of action is dependent on the rate of dissociation of various sized crystals at the subcutaneous site.

Novo Nordisk have developed this novel insulin analogue for treating diabetes, and Levemir (insulin detemir) was approved 2004 for treatment of diabetes 1 and 2 mellitus. Levemir is administered subcutaneously and is totally water-soluble avoiding some of the side effects that can occur with existing crystallized (cloudy) long-acting insulins.

Several open-labeled, randomized, multicenter trials have been conducted comparing the safety and efficacy of Levemir to isophane insulin human (NPH insulin) in patients with type 1 or type 2 diabetes mellitus. The onset of action of Levemir occurs within 1 h, which is faster than NPH insulin and its duration of action is up to 24 h. Levemir was well tolerated, the most common adverse effects reported during clinical trials being hypoglycemia, headache, dizziness, and injection-site reactions. Overall, Levemir was as effective as NPH insulin in maintaining overall glycemic control in adult patients with type 1 or type 2 diabetes mellitus [64]. Thus, the predictable and prolonged pharmacokinetic profile makes Levemir suitable as the basal component in a basal-bolus treatment regimen.

3.5. Albumin fusion proteins

The Albumin Fusion Technology yield albumin protein conjugates that are genetically engineered by splicing together the genes of the two molecules and expressing the albumin fusion proteins in yeast strains. Human Genome Sciences, Inc. has applied their technology to cytokines, primarily to interferon α-2b, interleukin-2 and granulocyte colony-stimulating factor, but also to bioactive peptides [22].

Clinically, the most advanced albumin fusion protein is Albuferon-α, a fusion protein of albumin and interferon α-2b that is currently in phase III studies for the treatment of hepatitis C. Interferon α-2b has molecular weight of ~19 kDa and despite its antiviral properties has a half-life in humans in the range of 2 to 3 h requiring frequent injections (daily or three times weekly). By genetically fusing recombinant interferon α-2b with recombinant human serum albumin a fusion protein with a molecular weight of 85.7 kDa is generated (see Fig. 9) that is being developed by Human Genome Sciences in collaboration with Novartis as a long-acting interferon for the treatment of chronic hepatitis C. Phase I and II studies demonstrated that Albuferon is generally well tolerated and was detectable in the blood of patients for up to 4 weeks following subcutaneous injection. As an example the blood concentrations of Albuferon at different dose levels after two subcutaneous injections are depicted in Fig. 9. The predicted half-life of ~140 h for Albuferon exceeds the published values for pegylated interferon-α [Pegasys (50 kDa) ~ 80 h, PEG-Intron (31 kDa) ~40 h [65]. In a phase II trial comparing Albuferon+ribavirin with Pegasys+ribavirin, the standard care for treating chronic hepatitis C, efficacy and adverse effects were comparable, but Albuferon was less frequently administered and there was an indication of reduced immunogenicity and less impairment of health-related quality of life. At present, the phase III programme is comparing the efficacy, safety and impact on health-related quality of life in combination versus Pegasys in combination with ribavirin.

A number of other albumin fusion proteins have entered early clinical trials. These include fusion proteins with low-molecular weight peptides such as β-natriuretic peptide and glucagon-like peptide 1, the latter following the same rationale as ConjuChem’s CJC-1131, as well as fusion proteins with other cytokines. Of interest for the oncologist is Albuluixin, an albumin fusion protein with recombinant interleukin-2 that has sown promising antitumor efficacy against murine renal cell carcinoma and melanoma [66].

4. Micro- and nanoparticles with albumin

4.1. Albumin microspheres

Albumin microspheres are generally prepared by chemical cross-linking or by addition of an organic solvent and stabilization at elevated temperatures. The size of the albumin microspheres which is
usually in the range of 1–100 μm is the decisive factor for the biodistribution characteristics of the albumin microsphere. Small microspheres (1–3 μm) are taken up by the reticuloendothelial system and accumulate in the liver and spleen as well as in solid tumors. Larger microspheres (>15 μm) will effectively target the capillary bed of the lungs. Albumin microparticles can carry therapeutic or diagnostic agents. The therapeutic approaches with albumin microspheres will not be discussed here since there was only one preliminary report of phase I trials with a cisplatin loaded or mitomycin C albumin microsphere in the mid 1980s and no candidate has currently reached an advanced preclinical stage. I refer the reader to two review articles on this topic [67,68].

For diagnostic applications, a 99mTc macroaggregated albumin has been developed that has found various clinical applications. 99mTc macroaggregated albumin is prepared by mixing a colloid solution of Sn(II) chloride with a solution of human serum albumin and subsequent labelling with sodium pertechnetate (99mTcO4− Na+). Depending on the amount and reaction time chosen, larger particles are formed in the range of 200–1000 nm (Albures, kit from Nycomed Amersham) [69] or smaller particles with an average size of 8 nm (Nanocoll, kit from Nycomed Amersham) [70] so the latter are rather nano- than microparticles. 99mTc macroaggregated albumin has been used diagnostically for various indications including lymphoscintigraphy [71] sentinel node detection in breast cancer [72] and other solid tumors [73,74], leg edema [75], protein-losing enteropathy [76], and rheumatoid arthritis [77].

### 4.2. Albumin nanoparticle technology (nab-technology)

American Bioscience, Inc. has developed a unique albumin-based nanoparticle technology (nab-technology) that is ideal for encapsulating lipophilic drugs into nanoparticles. The technology as such appears simple: the drug is mixed with human serum albumin in an aqueous solvent and passed under high pressure through a jet to form drug albumin nanoparticles in the size range of 100–200 nm that is comparable to the size of small liposomes. As an example, a model and electron micrograph of an albumin nanoparticle with paclitaxel (nab-paclitaxel) is shown in Fig. 10. nab-paclitaxel has an approximate diameter of 130 nm and has been extensively investigated, preclinically as well as clinically, and was approved in 2005 for the treatment of metastatic breast cancer. Preclinically, nab-paclitaxel has shown superior antitumor efficacy over paclitaxel in a number of human tumor xenograft models [78]. As an example, the antitumor effects of paclitaxel versus nab-paclitaxel is shown in Fig. 10 in the mamma carcinoma MX-1 model at equitoxic dose together with tumor uptake data in an equimolar comparison. Interestingly, paclitaxel accumulation in the subcutaneously growing MX-1 tumors was only 33% higher for nab-paclitaxel than that of paclitaxel when dosed at 20 mg/kg paclitaxel equivalents. However, when dosed at their respective maximum tolerated doses (30 mg/kg for nab-paclitaxel, 13.4 mg/kg for paclitaxel) there was a dramatic improvement in antitumor response for mice treated with nab-paclitaxel with complete remissions being achieved (see Fig. 10). When viewing such impressive

![Transmission Electron Microograph](image_url)

**Fig. 10.** Top: Model and transmission electron micrograph of nab-paclitaxel, a 130 nm albumin paclitaxel nanoparticle. Bottom: (left): Antitumor activity of nab-paclitaxel (ABI-007, 30 mg/kg/d) and Cremophor-based paclitaxel (13.4 mg/kg/d) in the MX-1 tumor xenograft model; (right): Intratumor paclitaxel concentrations following equal doses of ABI-007 and paclitaxel (20 mg/kg). Paclitaxel accumulation was 33% higher for ABI-007 than that of paclitaxel when equimolar doses were administered.
antitumor responses, one would have probably expected a larger drug targeting potential compared to a free drug, but this is obviously not the case, and the situation is quite comparable to the antitumor response and tumor uptake data for albumin-binding produgs such as DOXO-EMCH (see Section 3.1). Rather, it is the combination of an enhanced, albeit not dramatic improvement in tumor uptake for the respective albumin-based drug delivery system over the free drug when assessed at the MTD of the free drug and a favorable biodistribution and significant increase in its MTD that accounts for the striking difference noted between the two in preclinical tumor models. Based on the shift of the MTD of nab-paclitaxel (−2.2-fold) and of DOXO-EMCH (−4.5-fold) over the respective free drug in mice, the overall increase in drug tumor accumulation can be estimated to be −3– to 6-fold at an equitoxic comparison.

The enhanced uptake of albumin-based drug delivery systems in solid tumors is mediated by the pathophysiology of tumor tissue, characterized by angiogenesis, hypervasculature, a defective vascular architecture, and an impaired lymphatic drainage. In addition, scientists at American Bioscience, Inc. have collected data that accumulation of nab-paclitaxel is also due to transcytosis initiated by binding of albumin to a cell surface, 60-kDa glycophorin (gp60) receptor (albomind) as well as due to binding of albumin to SPARC (secreted protein acid and rich in cysteine). Albumin binds to the gp60 receptor, which in turn results in binding of gp60 with an intracellular protein (caveolin-1) and subsequent invagination of the cell membrane to form transcytotic vesicles, i.e. caveolae [78,79]. In a preclinical study using radiolabeled paclitaxel, endothelial binding and transport of nab-paclitaxel was significantly increased compared to free paclitaxel and endothelial transcytosis was completely inhibited by methyl 8-cyclohexyl, a known inhibitor of gp60/ caveolar transport [78]. Furthermore, preliminary evidence suggests that tumor accumulation of nab-paclitaxel may be facilitated through binding to SPARC, an extracellular matrix glycoprotein that is overexpressed and associated with poor prognosis in a variety of cancers including breast cancer [80].

The encouraging preclinical data made nab-paclitaxel (Abraxane) an obvious candidate for clinical development. In addition, Abraxane avoids the use of cremophor. Due to its extremely low water-solubility, paclitaxel is formulated in polyethoxylated castor oil (Cremophor EL) and ethanol. Clinically, however, prolonged infusion times (3 h) and premedication with corticosteroids and antihistamines are required to reduce the risk of hypersensitivity reactions. Cremophor-based paclitaxel can also cause neutropenia, and prolonged, sometimes irreversible, peripheral neuropathy, which may be associated with axonal degeneration [81,82].

Clinical studies with Abraxane were carried out without any premedication and, not surprisingly, have not shown any hypersensitivity reactions. After successful phase I and II studies [83,84], a large phase III study in metastatic breast cancer was carried out that after completion paved the way for approval of Abraxane by the FDA for the treatment of patients with metastatic breast cancer who have failed combination therapy [85]. In this study, patients were randomly assigned to 3-week cycles of either nab-paclitaxel (260 mg/m² IV over 30 min every 3 weeks) or Cremophor-based paclitaxel (175 mg/m² IV over 3 h with premedication) (n = 225). Treatment with nab-paclitaxel resulted in increased response rates compared with Cremophor-based paclitaxel (33% versus 19%) and prolonged time to tumor progression (23.0 versus 16.9 weeks). In addition, survival was increased in patients receiving nab-paclitaxel as ≥2nd-line therapy, with risk of death reduced by 28% although this difference was not statistically significant.

Despite a 1.5-fold higher paclitaxel dose administered in nab-paclitaxel, the incidence of grade 4 neutropenia was significantly lower with nab-paclitaxel than with Cremophor-based paclitaxel (9% versus 22%). At the higher dose, grade 3 sensory neuropathy occurred more frequently with nab-paclitaxel than with Cremophor-based paclitaxel (10% versus 2%), but neuropathy improved rapidly to grades 2 or 1 and was easily managed with treatment interruption and dose reduction. The rapid resolution of sensory neuropathy in the nab-paclitaxel group could be explained by the fact that nab-paclitaxel avoids the prolonged neuropathy associated with axonal degeneration caused by Cremophor [82].

Preliminary results from an open-label multicenter study of 210 Chinese patients with metastatic breast cancer suggest that nab-paclitaxel (260 mg/m² IV over 30 min every 3 weeks) provides higher response rates and longer time to tumor progression without increased toxicity compared to solvent-based paclitaxel (175 mg/m² IV over 3 h every 3 weeks) [86].

Following its approval in January 2005, Abraxane is being further evaluated for adjuvant, neoadjuvant, and first-line treatment in breast cancer as well as for other indications such as non-small cell cancer, ovarian cancer and pancreas cancer. Furthermore, several pipeline products based on the nab-technology are under development, e.g. with docetaxel and rapamycin, and have entered or are advancing to clinical trials (www.americanbiosciences.com).

5. Summary and outlook

Albumin is the chief circulating protein in the blood circulation and a transport protein per se for a number of endogenous and exogenous compounds. For the protein chemist, albumin is not a standard protein since it is extremely robust towards pH, temperature and organic solvents and can be stored as a 5 or 20% solution for many years. The different uses of albumin as a drug carrier that have emerged in the past 10 years are fascinating and range from extending the half-life of therapeutically active proteins and peptides (e.g. Albuderon, Levenir and CJ-1134-PC), and drug targeting (e.g. MTX-HSA, INNO-206 or Abraxane). The development and market approval of the paclitaxel albumin nanoparticle, Abraxane, can be viewed as a landmark not just for albumin-based drug delivery technology but also for nanomedicine with annual sales reaching approximately US$300 Mio in 2007, only 2 years after its launch. Other drug formulations in which the drug is bound ex vivo or in vivo to albumin are advancing to phase II and III trials, respectively. In addition, several pipeline products are under development with the various platform technologies that are based on albumin as a drug carrier and that were outlined in this review. It is my opinion that the use of albumin as a drug carrier will not end here. There are some exciting avenues for the medical application of albumin that have not been fully explored such as its use in photodynamic therapy [87,88], as a transport protein for metal complexes [89,90], its use as an anti-HIV agent [91–93], the use of cationized albumin as a drug carrier for blood brain-barrier transport [94,95], albumin as a gene delivery vector [96,97], albumin microbubbles that release the drug after destruction by ultrasound [98], and the development of tetraphenylporphino-iron(II) bound to albumin as an artificial blood substitute [99,100]. Finally, the development of subcutaneously and orally applicable albumin-binding derivatives will be an important research area as soon as a clinical proof of concept has been established for intravenously administered counterparts.

References


