Bioactive albumin-based carriers for tumour chemotherapy

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Abstract

Proteins are posed as the natural counterpart of the synthetic polymers for the development of drug delivery systems and few of them, have been regarded safe for drug delivery purposes by the United States Food and Drug Administration (FDA). Serum albumin is the most abundant protein in human blood. Interest in the exploration of pharmaceutical applications of albumin-based drug delivery carriers, especially for the delivery of chemotherapeutic agents, has increased in recent years. Albumin has several advantages over synthetic polymers, as it is biocompatible, biodegradable, has low cytotoxicity and has an excellent binding capacity with various drugs. Micro- and nano-carriers not only protect active pharmaceutical ingredients against degradation, but also offer a prolonged release of drugs in a controlled fashion. Since existing tumour chemotherapeutic agents neither target tumour cells, nor are they specific to tumour cells, a slow release of drugs from carriers would be beneficial in targeting carcinogenic cells intracellularly. This article aims at providing an overview of pharmaceutical applications of albumin as a drug delivery carrier in tumour chemotherapy.

Keywords: Albumin; cancer; chemotherapy; drug delivery; microspheres; nanoparticles
INTRODUCTION

The use of natural bioactive and biodegradable polymers for delivering drugs to their target sites continues to be an area of great interest, despite the advent of synthetic polymers [1]. Natural proteins are being widely modelled by counterpart synthetic polymers in the development of drug delivery systems and are generally regarded as safe by the United States Food and Drug Administration for drug delivery.

Synthetic and natural polymers are currently in use for the preparation of drug carriers; both polymer systems have some advantages and disadvantages associated with them. Ideally, synthetic and natural polymers should be biodegradable in the body to prevent accumulation in various organs and potentially cause unwanted side effects after chronic use. Synthetic polymers offer “customisation” that allows for preparation of polymers with desired functions. A major disadvantage of the synthetic polymers is the cost and time associated with their synthesis. On the other hand, most of the natural polymers are biodegradable and biocompatible besides being relatively inexpensive. Furthermore, the degradation products of naturally occurring polymers can be metabolised and/or can easily be cleared from the body. Nonetheless, there is a potential risk of viral infection and lack of uniformity among various product batches.

Over the past decades, proteins have emerged as versatile carriers for drug targeting and for improving the pharmacokinetic profiles of various protein-based drugs, used in many clinical settings, from diagnoses to the treatment of several diseases, including cancer, diabetes and inflammatory disorders [2, 3]. Albumin has a long history of pharmaceutical use in delivering drugs to their target sites. Albumin is predominately derived from human plasma, although both time-expired blood, and in some countries, even placental materials were used as a source in the past. The amount of albumin sourced from time-expired blood has declined markedly due to the frequent availability of red cells separated from the whole blood. Moreover, albumin sourced from placental material has been deemed unsafe for use as a delivery vehicle with regards to possible transmittance of viruses and also faces difficulties in ensuring donor traceability [4]. Other sources of albumin that are routinely used as carriers for drug delivery include bovine serum albumin (BSA) and egg albumin (ovalbumin; OVA) [5, 6].

Human serum albumin (HSA) is the major and the most abundant component of the plasma proteins, with a concentration of approximately 35 - 50 mg/mL, thus comprising 60% of the total plasma proteins [4, 7]. HSA is a single chain, macromolecular protein comprising of 585 amino acids, with a molecular weight of 65 kDa (Fig. 1), and has a biological half-life of 19 days [3]. It is synthesised in the liver polysomes, bound to the
endoplasmic reticulum, at a rate of 9 - 12 g/day [8, 9], representing about 25% of total hepatic synthesis. Half of the total hepatic synthesis is comprised of secreted proteins, of which 40% of albumin is present in the plasma and the other 60% in the extracellular space [10]. Amino acid deficiencies (particularly leucine, arginine, isoleucine and valine) as a result of malnutrition can limit the albumin production, although these are rarely observed clinically [9]. It has the primary function of modulating the colloid osmotic pressure of the blood and plays a major role in controlling the movement of fluids between body compartments. Albumin binds weakly and reversibly to both cations and anions despite being strongly negatively charged. It, therefore, functions as a circulating depot and transports molecules for a large number of metabolites, including fatty acids, thyroxine, bilirubin and amino acids [9]. Furthermore, it binds with many drugs, for example penicillin, sulfonamides, indole compounds and benzodiazepines. It is also involved in the binding and transportation of certain metal ions, such as copper, nickel, calcium and zinc in the blood [3].

Fig. (1). Crystal structure of HSA [11], reproduced by permission of the Oxford University Press.

HSA therapy has been considered in various pharmacological situations, including shock, burns, hypoalbuminemia, surgery or trauma, cardiopulmonary bypass, acute respiratory distress and haemodialysis [12]. Since HSA is blood derived, the risk of transmitting possible viral/prion contaminants exists [13]. Recombinant HSA (Recombumin) has been developed as an alternative to the blood derived HSA. It is a genetically engineered protein, expressed in yeast cells and has demonstrated similar safety, tolerability, pharmacokinetics and pharmacodynamics profiles to natural HSA [14].
Albumin is stable in the pH range of 4 - 9 and can be heated at 60°C for up to 10 h without deleterious effects. Its solubility in ethanol is 40%. The most important property of albumin is its preferential uptake in tumour and inflamed tissue, which makes it as an ideal candidate for use in drug delivery [3]. This article, therefore, gives an overview of the different pharmaceutical technologies that currently make use of albumin as a carrier for tumour chemotherapeutic agents, including micro- and nano-carriers.

**Tumour chemotherapy and its limitations**

Cancer is an extremely complex disease that defines a group of disorders, characterised by the continuous, indefinite proliferation of cells [15]. Tumours can be benign (localised) or malignant (metastasised, i.e. spread to other parts of the body). Through the archives of history, the fear of cancer has affected the human race. Regardless of excellent advances in cancer treatment, it is still one of the prime roots of deaths worldwide, and it remains a challenge to scientists. More than 200 different types of cancers have been reported globally [16]. In the United States (US), cancer kills one of every four persons and affects both genders and all age groups. In 2013, about 1,660,290 new cancer cases and 580,350 of cancer deaths were estimated in the US by the Cancer Statistics Review [17].

The three main therapies used to treat tumours are (a) surgical excision, (b) irradiation and (c) chemotherapy. Comparative values of these three approaches depend on the type of tumour and the stage of tumour development. When the disease is in its initial stages and the patient is at lower risk, it is often treated with surgery alone, although in many cases, a combination of therapies is required. In the case of a malignant tumour, systemic therapy is required as a major therapeutic approach, as delivery through the blood stream encourages the access to promulgated tumour sites. Systemic remedies include hormonal therapy, targeted therapy and chemotherapy. A major and the most common therapeutic approach for the treatment of localised and metastasised tumour is chemotherapy, which is used alone, or in combination with the other types of therapies [18].

Chemotherapy had been used to treat cancer since the beginning of 20th century. Different methods had been developed for the screening of chemicals by utilising the transplantable tumour cells in rodents. Chemotherapy has changed much over time and currently, different molecular abnormalities are being used to screen for potentially new drugs and also for more targeted treatments [19]. The aims of cancer chemotherapy treatments include cure, control and palliate cancer.
All the currently available chemotherapeutic agents attack tumours at the cellular level by interfering with the processes, or substances that are involved in the uncontrolled replication of cells. Cytotoxic drugs induce cell death through apoptosis, either by directly restricting the DNA, or by attacking the key proteins involved in cell replication. The classification of chemotherapeutic agents depends upon their cell cycle specificity, an important criterion, as it is helpful in the scheduling of drugs and combination therapies. The therapeutic agents that affect a specific phase of the cell cycle usually have a dose response effect up to a threshold, at which point dose augmentation does not improve cell death. In contrast, non-phase specific therapeutic agents show a dose response relationship, and usually the threshold dose is restricted by the toxic effect on normal cells [20]. Some limitations are, however, related to the use of conventional chemotherapy. Firstly, most of the chemotherapeutic agents of either plant source or synthetic origin are hydrophobic and require solvents for formulating the dosage form, which may lead to severe toxicity. Secondly, antineoplastic agents lack selectivity for tumour cells and cause significant damage to rapidly dividing normal cells [21, 22]. Finally, drug resistance occurs in most tumours treated with chemotherapy. Since normal tissues possess intact genetic machinery, they do not develop resistance. Sensitive cancer cells may develop drug induced mutations, which may later develop drug resistance [23].

**Targeting tumorous cells**

Chemotherapy aims at utilising cytotoxic drugs to destroy tumour cells, without exerting any pharmacological effects on normal cells through a biological response. The more selective the drug is towards targeting the tumour, the more effective it would be and the more limited any undesirable drug distribution to critical normal tissues, resulting in fewer adverse effects [24]. The ultimate goal of chemotherapy, therefore, is to improve the efficacy of the anticancer agent and to reduce its toxicity. This can be achieved by optimising the selectivity of a chemotherapeutic agent to specifically target the tumour mass and by maintaining a local concentration long enough to obtain an optimal therapeutic effect [25]. It has been suggested that albumin accumulates in malignant and inflamed tissue, due to permeable and defective capillaries, combined with an absent, or faulty lymphatic drainage system in these unhealthy tissues [26]. According to Noguchi et al., the enhanced uptake of macromolecules, including albumin, by tumour tissues could not be merely explained by an enhanced permeability of the vascular system, as this would also affect smaller molecules in a similar manner. Additionally, the enhanced uptake of macromolecules could also be caused by a reduced clearance from the tumour when the molecular weight exceeds 40 kDa [27]. The uptake of albumin by tumorous cells can be made visible in preclinical models by injecting Evans blue dye that rapidly and securely binds to circulating albumin,
causing subcutaneously growing tumours to turn blue within a few hours post-injection (Fig. 2) [3]. Thus, enhanced permeability and retention (EPR) are both responsible for the long residency of albumin in tumourous cells. These properties of albumin make it a potential candidate for the diagnosis and treatment of tumours.

![Fig. 2](image)

**Fig. (2).** Accumulation of Evans blue albumin complex in subcutaneously growing sarcoma 180 tumours over 72 h [3], reproduced by permission of Elsevier.

**Albumin-based pharmaceutical technologies in tumour chemotherapy**

Albumin is a versatile macromolecule, with a number of pharmaceutical applications. It is routinely used as a stabilising constituent in vaccines, recombinant therapies and coatings for medical devices. Drug formulations, using albumin as a carrier, show promise of acquiring beneficial stabilising effects physiologically, because of its known antioxidant properties [13]. Albumin is emerging as a viable drug carrier for administering higher doses of a chemotherapeutic agent to cancer patients, without increasing the side effects, whilst improving anticancer efficacy [28]. This section aims at reviewing most commonly used albumin-based pharmaceutical strategies intended for tumour chemotherapy.
Albumin-based micro-carriers

Micro-carriers are small particulate systems that carry dispersed drug particles either in solution or crystalline form, and are usually in the range of 0.1 micron to several hundred microns in size, depending on their intended use [29]. Micro-carriers, such as microspheres and microcapsules are considered valuable drug delivery systems, offering unique properties, such as surface-to-volume ratios that favour exchange between their load and the physiological environment after administration [30]. A number of bioactive agents, including synthetic drugs, proteins, and cells have been encapsulated in micro-carriers, made from biocompatible and biodegradable natural, or synthetic polymers in the form of either particles, or capsules for a wide range of applications, including fundamental studies of biological systems, drug delivery, tissue engineering and the immuno-isolation of cells. The interaction and release kinetics of various drugs can be precisely controlled by specially designed micro-carriers to fully achieve their goals [30, 31]. The next section reviews albumin-based microspheres intended for tumour chemotherapy.

Microspheres

Solid, approximately spherical particles, ranging between 1 - 1,000 µm in size are termed as microspheres [32]. Microspheres, prepared from biopolymers and their synthetic counterparts have been widely studied for their application in cancer therapies [33, 34]. Bioactive and biodegradable microspheres, especially those using albumin as a polymer matrix, have attracted much attention over the past few decades [33]. They have been widely used in sustained drug delivery systems as drug carriers for site-specific and for the remote site release of drugs [35]. They have been reported as relatively non-toxic, non-immunogenic, comparatively easy to formulate over a wide range of particle sizes. These microspheres carry reactive groups (amino and carboxylic groups) on their surfaces that can be used for radionuclide-, chelator- and antibody binding and/or for other surface modifications [36-39]. Usually, HSA, BSA and OVA are the sources of albumins used in the preparation of such microspheres [1].

The concept of using albumin microspheres in drug delivery systems was introduced by Kramer [40]. Later, Kramer and Burnstein studied the uptake of suspended HSA microspheres, loaded with a radiolabelled anticancer agent, i.e. 8-¹⁴C-labelled-6-mercaptopurine (6-MP-8-¹⁴C), by several tumour cell lines. The authors reported that albumin microspheres had been phagocytosed by tumour cell lines, hence proving that these carriers are a suitable vehicle for delivering drugs to malignant cells, while preventing these agents from producing toxic effects in non-phagocytic tissues [41]. This pioneering work by Kramer and Burnstein [41] was
successfully extended by Widder et al. [42]. As proof of this concept, they formulated albumin-based magnetic microspheres, loaded with doxorubicin (DOX) at a dose level of either 0.5 mg/kg, or 2.5 mg/kg for their targeted and selective localisation in Yoshida sarcoma tumours. Drug loaded microspheres were injected proximal to the tumour via the ventral caudal artery in Yoshida sarcoma bearing rats. Out of the 22 animals receiving DOX loaded magnetic microspheres, 17 animals showed total histological remission of the tumour. The remaining animals demonstrated significant reduction in tumour size. This study, therefore, further strengthened the applicability of albumin microspheres as delivery vehicles for anti-tumour agents.

Since the pioneering work of Kramer [40], followed by Kramer and Burnstein [41], and later by Widder et al. [42], many successful studies on albumin microspheres focusing on targeting tumorous cells have been documented by several researchers, such as the work of [43-47].

Melanoma is the most dangerous form of cutaneous cancer and accounts for one third of all cancers in the United States, with the incidence having risen sharply over the last few decades [48]. Bhowmik et al. [49] formulated a spray-dried microparticulate melanoma cancer vaccine, intended for delivery through the transdermal route. Vaccine was administered; using a microneedle based Dermaroller®, a commercial device available for cosmetic purposes. Microneedle is a minimally invasive technique, offering the painless administration of drugs and proteinaceous macromolecules that would otherwise penetrate the skin with difficulty through passive diffusion. The tumour challenge study, during which tumour cells had been injected in both immunised and control group of animals, revealed that no measurable tumour growth had occurred for 35 days after tumour injection in the vaccinated group of animals. Contrary, the non-vaccinated control group of animals had developed a palpable tumour at around 11 days, reaching a maximum tumour size by day 21, after injection of the tumour cells.

Nano- and microparticles that are used to deliver the drug and also assist in diagnosing, dubbed as theragnostic nanoparticles, are becoming increasingly popular [50-52]. Kolluru, et al. [51], developed albumin based nanoparticles as tumour theragnostic agent by entrapping doxorubicin and a near infrared (NIR) dye, indocyanine green (ICG). Win et al. [53], prepared polymeric nano- and microparticles and reported that microparticles accumulated selectively in cancer cells and not in normal cells, thus permitting targeting cancer cells for site specific drug delivery.

The poor aqueous solubility of chemotherapeutic agents significantly limits their delivery and hence their efficacy. Albumin-based microspheres as delivery vehicles can offer an attractive solution to countering
this problem. Paclitaxel, a lipophilic anti-tumour drug with poor aqueous solubility, is isolated from the bark of *Taxus brevifolia* and is used to treat a wide range of solid tumours, including breast, non-small lung cell, epithelial and ovarian cancers [54, 55]. Grinberg *et al.* prepared BSA microspheres, loaded with paclitaxel by using a sonochemical reaction procedure, followed by assays for chemical and biological activities. This procedure did not compromise the drug, which had been successfully encapsulated in the BSA microspheres [56]. Results showed that BSA microspheres, loaded with paclitaxel had caused an increase in tumorous cell deaths, compared to the control.

Since microspheres offer unique surface properties, the coupling of microspheres with antibodies is a promising approach for avoiding toxic effects of loaded drugs and in improving their efficacy towards tumour cells [57-59]. This feature was practically demonstrated by Grinberg *et al.* in their most recent work [60]. They encapsulated the anticancer drug, gemcitabine (Gem) in BSA-based microspheres as a delivery vehicle, containing the anti-epidermal growth factor receptor (EGFR) antibody as targeting agent. Administration of these loaded BSA microspheres (BSA-Gem-EGFR) showed marked inhibition of pancreatic cancer cells (AsPC1), compared to those cells treated with BSA microspheres only, BSA with gemcitabine (BSA-Gem), or free Gem (Fig. 3).

![Comparison of loaded and unloaded BSA microspheres showing inhibition of cell proliferation observed after 48 h of incubation of AsPC1 pancreatic cancer cells.](image)

*Fig. (3).* Comparison of loaded and unloaded BSA microspheres showing inhibition of cell proliferation observed after 48 h of incubation of AsPC1 pancreatic cancer cells [60]. Reproduced by permission of John Wiley and Sons.

Another interesting technique for targeting the tumour site is the use of externally controlled magnetic drug delivery systems [61]. Magnetic microspheres, infused into the body, can be escorted to the disease site up to several-fold concentrations from the blood circulation, using an effective external magnet [62]. Enriquez *et al.* [63] demonstrated that the preparation of albumin-iron oxide magnetic microspheres had been capable of
efficiently delivering in vivo a tumour chemotherapeutic agent, i.e. sulforaphane, a histone deacetylase inhibitor, for an extended period of time. In vitro studies in B16 melanoma cells revealed that about 13 - 16% more inhibition of cell viability had occurred when either 30 μM, or 50 μM of sulforaphane was used, together with iron oxide in the BSA microspheres. Data from in vivo studies in C57BL/6 mice revealed that the magnetic microspheres (localised at the tumour site with the aid of a strong magnet) had inhibited 18% more tumour growth, compared to free sulforaphane in solution.

**Albumin-based nano-carriers**

Nano-carriers are particles in the range of 1 - 100 nm in dimension [64]. Pharmaceutical nano-carriers include polymer drug conjugates, polymeric nanoparticles, lipid based carriers (such as liposomes and micelles), dendrimers and carbon nanotubes, amongst others, and possess the ability to carry drugs, proteins, imaging agents, photothermal ablation of tumours, radiation sensitisers, detection of apoptosis, and sentinel lymph node mapping [65]. Nano-carriers are widely investigated for possible application in cancer treatments, of which some have already been approved for clinical use, while others are still being investigated. These carriers face several barriers (mucosal barriers and non-specific uptake), before reaching the target site of action. Drug targeting of tumorous cells is often classified as passive, or active targeting, depending on the use of specific tumour pathophysiology and the interaction of the carrier and target cells [64, 66].

To date, these nano-carriers have been developed by using polymers (both synthetic and natural), surfactants, lipids and proteins, amongst others. Proteins, such as gelatine, collagen, casein, whey protein and albumin have been studied with regards to the delivery of drugs, nutrients, bioactive peptides and probiotics. Compared to synthetic polymers, proteins offer several advantages, such as absorbability and low toxicity.

Currently, three distinct types of albumins, i.e. (1) OVA, (2) BSA and (3) HSA are used in the preparation of nano-carriers [67]. This section further reviews the uses of albumin in the preparation of different nano-carriers, specifically used for targeting different types of tumours.

**Drug-albumin conjugate**

Polymer-drug conjugates are widely used to increase the solubility of drugs, to protect drugs from their surrounding environments and to increase the circulation time of drugs. The concept of polymer anticancer conjugates was introduced by Ringsdorf in 1975 [68]. Since then, their design and mechanisms of action have been well understood by scientists. A polymer anticancer conjugate consists of a natural or synthetic, water
soluble, polymeric carrier (usually of 10,000 - 100,000 Da), a biodegradable linkage or spacer, and an anti-tumour agent. To improve their specificity with regards to the targeted tumour cells and their local concentrations, a targeting moiety can also be attached to these conjugates (Fig. 4) [66].

![Diagram](image)

**Fig. (4).** The various components of a polymer-drug conjugate for targeted cancer therapy.

Because of the low aqueous solubility of paclitaxel, its conventional formulations require the use of cremophor EL (polyethoxylated castor oil) and ethanol as solvents. Paclitaxel causes severe hypersensitivity reactions upon administration. Different approaches are adopted to improve the solubility and efficacy of paclitaxel and to reduce its toxicity. One approach is to conjugate drugs into a stable macromolecular carrier. It is known that macromolecules, such as albumins, globulins and synthetic polymers accumulate in tumour cells, due to the enhanced permeation retention effect (EPR) [69]. Additionally, they can improve the pharmacokinetics [70] and impede cellular mechanisms involved in the development of drug resistance [71]. Albumin is an attractive polymer to conjugate with paclitaxel, as it is highly water soluble and known for its accumulation in tumorous tissues. To address the issue of solubility, toxicity and efficacy, Dosio *et al.* covalently attached paclitaxel to HSA in different ratios to prepare conjugates in which each HSA was attached to 6 and 30 molecules of paclitaxel each. In the conjugated form, paclitaxel maintained its high cytotoxicity, with efficient cell binding and internalisation, followed by its release in the targeted tumour cells. These paclitaxel-albumin conjugate also improved the therapeutic efficacy of the anticancer agent, as shown by the high area under the curve (AUC) values of the conjugates. The conjugates also strongly lowered the toxicity profile of the free drug, since even a high administration of a 70 mg/kg dose of the conjugate to mice had shown minimal side effects. This conjugate hence acted as depot preparation, while simultaneously decreasing the toxicity and improving the therapeutic efficacy of the active [69]. In another investigation, to further improve the solubility, circulation time and stability of paclitaxel HSA, Dosio *et al.* covalently bound a monomethoxy poly(ethylene glycol) (mPEG) to albumin. This conjugate maintained the cytotoxicity of paclitaxel, its internalisation and the targeted release of the drug in cancer cell lines. *In vivo* studies showed reduced
localisation in the liver and spleen, indicative of the prolonged circulation of these carriers, in particular, the one formed by 5 kDa PEG [72]. In their most recent work, Dosio et al. attached a targeting moiety to make paclitaxel specific for cancer cells bearing folate receptors. The drug-HSA conjugate was further equipped with folic acid through an extended PEG spacer. In vitro studies on folate receptor positive human nasopharyngeal epidermal carcinoma KB cell lines showed increased binding, cellular uptake and toxicity, due to the folic acid [55]. In another interesting study, Guo et al. attached a low molecular weight protamine (LMWP) as cell penetrating peptide to BSA, thus enhancing tumour cell penetration. Approximately eight DOX molecules were conjugated to the polymer back bone through cleavable disulphide bonds, causing the release of the prodrug, due to these conjugates also being responsive to the highly reducing environment in the cytosol of the tumour cells. This new conjugate showed favourable results in different cell lines, compared to DOX alone.

DOX alone was unable to destroy the drug-resistant MCF-7/ADR cells, even at concentrations up to 6 µg/ml and cell viability of 80%. In comparison, LMWP–BSA–DOX showed considerable activity towards the drug-resistant cells, with a 50% inhibitory concentration (IC50) of 4.8 µg/ml. In vivo studies also showed the ability of conjugates to shrink tumour size, as illustrated in Fig. 5 [73].

Fig. (5). Comparison of anti-tumour efficacy of LMWP–BSA–DOX in tumour bearing mice: (A) tumour growth trend during the course of treatment; (B) tumour growth inhibition rate at the end of the investigation; (C) representative tumours after treatment [73]. Error bars represent the mean with SD. Reproduced by permission of The Royal Society of Chemistry.
Taheri et al. conjugated methotrexate to HSA through a carbodiimide reaction, followed by cross linking with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC) to form nanoparticles [74]. This conjugate was then cross linked to form nanoparticles, to which biotin molecules were used to modify their surfaces, so as to target cells overexpressing biotin receptors. In continuation of their study, Taheri et al. conjugated methotrexate to HSA and cross linked them to form nanoparticles, after which trastuzumab molecules were added to modify their surfaces. Trastuzumab is an anti-HER2 monoclonal antibody, commonly used in the treatment of metastatic breast cancers. HER2 is epidermal growth factor receptor and is overexpressed in 20 - 30% of human breast cancers. It was found that targeted nanoparticles were more effective in treating HER2 positive cells, compared to non-targeted particles and free methotrexate [75]. Manoochehri et al. first developed docetaxel nanoparticles of poly-lactic-co-glycolic acid (PLGA), after which their surfaces were conjugated with HSA. A cytotoxicity evaluation showed that the IC$_{50}$ of HSA conjugated PLGA nanoparticles (5.4 $\mu$g) was considerably lower than both free docetaxel (20.2 $\mu$g) and unconjugated PLGA nanoparticles (6.2 $\mu$g) [76].

To summarise, the above outcomes demonstrated that the conjugation of albumin to anticancer drugs had resulted in an improvement in the free drug’s solubility, its stability, tumour uptake by EPR, and its $\textit{in vitro}$ and $\textit{in vivo}$ efficacy against cancer cells. Furthermore, the active targeting of these conjugates showed improved results, compared to non-targeted conjugates.

**Nanoparticles**

Generally, microscopic particles in the range of 1 - 100 nm are regarded as nanoparticles. For drug delivery purposes, however, submicron ($<$ 1 $\mu$m) colloidal particles, to which a drug is adsorbed, dissolved from, or dispersed throughout the matrix, or is present in an aqueous or oily core surrounded by a shell-like wall, or is covalently attached to the surface, are also referred to as nanoparticles [77]. This is due to the relatively larger surfaces required to load a sufficient amount of drug during drug delivery. Fundamentally, a nano-delivery system, therefore, consists of a drug and a carrier. Drugs can sometimes even be formulated at nanoscale to act as their own carriers. Nanoparticles can be formulated using material from natural origin, such as phospholipids, lipids, albumin, lactic acid, dextran and chitosan, or from chemical origin, e.g. various polymers, carbon, silica and metals [78].

This section focuses on albumin nanoparticles, developed particularly for cancer chemotherapy. Table 1 summarises some anti-tumour agents encapsulated in albumin nanoparticles. Albumin is an ideal material for
nano-encapsulation of chemotherapeutic drugs, because most drugs show high binding properties, and due to these particles being biodegradable, easy to prepare and reproducible [67]. Albumin nanoparticles have been widely investigated for use with paclitaxel. The American Bioscience, Inc. developed an albumin-based nanoparticle technology, called “nab-technology”, in which HSA and drugs in aqueous solution are passed through a jet under high pressure to form drug albumin nanoparticles in the range of 100 - 200 nm. The FDA had approved 130 nm sized nab-paclitaxel in 2005 under the trade name of Abraxane®, for the treatment of breast cancer. During their clinical trials, Abraxane® showed superior activity, compared to cremophor base paclitaxel formulations. It was found that the accumulation of nab-paclitaxel had been due to trans-cytosis that had occurred when albumin had bound to the endothelial cell surface receptor, 60-kDa glycoprotein (gp60) (also known as albondin), after which the albumin then bound to secreted protein acid, rich in cysteine (SPARC), as illustrated in Fig. 6 [3, 79, 80].

![Diagram of internalisation of nab-paclitaxel nanoparticles through the gp60 trans-cytosis pathway and the subsequent binding to SPARC in the tumour’s extracellular matrix.](image)

**Fig. (6).** Schematic representation of the internalisation of nab-paclitaxel nanoparticles through the gp60 trans-cytosis pathway and the subsequent binding to SPARC in the tumour’s extracellular matrix. High affinity of albumin for gp60 and SPARC were responsible for its accumulation in cancer tissues in high concentration [80], reproduced by permission of Elsevier.

**Table 1.** List of some other active molecules being encapsulated in albumin nanoparticles for use in cancer chemotherapy
<table>
<thead>
<tr>
<th>Drug molecule</th>
<th>Purpose</th>
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<tbody>
<tr>
<td>Cisplatin</td>
<td>Folate conjugated HSA magnetic nanoparticles for combining targeted drug delivery with thermotherapy [78].</td>
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<tr>
<td>Curcumin</td>
<td>Curcumin loaded serum albumin nanoparticles for intravenous administration have shown improved water solubility (300 times, compared to pure curcumin) and bioavailability [79].</td>
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<tr>
<td>10-hydroxycamptothecin</td>
<td>10-hydroxycamptothecin loaded HSA have shown potential for liver targeting [80].</td>
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<tr>
<td>Sunitinib</td>
<td>Targeted sunitinib loaded HSA nanoparticles. EGFR antibody, called anti-EGFR-1 nanobody (EGa1), is used to target ligands. These particles have shown 40 times higher binding to EGFR positive cells than non-targeted particles [81].</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>HSA particles for the targeting of EGFR positive colon carcinoma cells [82].</td>
</tr>
<tr>
<td>Antisense oligonucleotides</td>
<td>Albumin particles, loaded with antisense oligonucleotides, used in gene therapy [83].</td>
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Abraxane® reduces the side effects, infusion time, pre-treatment with steroids and antihistamines associated with cremophor based paclitaxel, while permitting a higher dose (260 mg/m² compared to 175 mg/m²) that would reduce the risk of drug resistance development. Karmali et al. [88] modified these nab-paclitaxel nanoparticles with a homing device to increase their effectivity. Two peptides, i.e. cysteine-arginine-glutamic acid-lysine-alanine (CREKA) and cys-gly-asn-lys-arg-arg-gly-cys (LyP-1) were used as targeting moiety. CREKA is a pentapeptide that binds to clotted plasma proteins, specifically present in tumour vessel walls and interstitial tissues. LyP-1 is a cyclic nine amino acid peptide that binds to protein, known as p32, or gC1qR receptor. This protein is specific to tumour cell and lymphatics.

When these modified particles were injected intravenously into mice, bearing MDA-MB-435 human cancer xenografts, fluorescein-CREKA-abraxane accumulated in tumour blood vessels, while fluorescein-LyP-1-abraxane localised in the extravascular space, presumably having lymphatic vessels and lesser blood vessels.
Tumour treatment results showed that LyP-1-abraxane had significantly improved the efficacy of Abraxane®, compared to CREKA-abraxane [88].

Like paclitaxel, DOX is also widely investigated for use with albumin particles. DOX is antineoplastic and its use is limited by its high toxicity, especially cardiotoxicity. Nanoparticle formulations in the range of 100 - 500 nm can target cancer tissues through a passive tumour targeting process, due to the EPR effect [26] and can also overcome drug resistance. In comparison with other particles, HSA nanoparticles carry functional groups (i.e. amino- and carboxylic groups) that can be used for further surface modifications [89]. Dreis et al. incorporated DOX in HSA particles, which are in the 150 - 500 nm range. They incorporated cytotoxic drugs through adsorption to the nanoparticles’ surfaces, or by incubating the drug with matrix protein, prior to desolvation and cross linking. The loading efficiency was in the range of 70 - 95%, whilst the drug contents did not affect the overall tumour diameter. Compared to DOX solutions, these particles showed better anti-tumour activity [90]. These earlier studies had only aimed at incorporating DOX into albumin particles, whereas more recent studies have aimed at making them more target specific by attaching different ligands to them. Wagner et al., for example, developed DOX loaded HSA nanoparticles and their surfaces were modified with a monoclonal antibody. DI17E6 monoclonal antibody is specific for αvβ3 integrin. αvβ3 is overexpressed in a variety of cancer cells and plays an important role in angiogenesis. DI17E6 has also shown anti-tumour activity against different cancers. These targeted DOX loaded nanoparticles showed increased cytotoxic activity in αvβ3 positive melanoma cells, compared to the free drug [91]. This was attributed to the incorporation of drugs in the carriers and to their modification with DI17E6, which has multiple functions, like the inhibition of melanoma growth, angiogenesis and the directing of carriers to specific cells. In another study, Bae et al. prepared DOX loaded nanoparticles and modified their surfaces with tumour necrosis factor (TNF) related apoptosis-inducing ligand (TRAIL) and transferrin. TRAIL is a ligand for TNF receptors and is overexpressed in the majority of cancer cells [92]. This protein can selectively bind to two death receptors (DR4, DR5) and initiates the extrinsic death pathway through caspase dependent apoptotic signal transduction. Although TRAIL is considered a safe anticancer drug [93], some cancer cells resist TRAIL induced apoptosis. Transferrin is a ligand for transferrin receptors, overexpressed in cancer cells. The strategy by Bae et al. to combine DOX, TNF and transferrin in one albumin particle can overcome the mechanism of resistance found in resistant cells, as illustrated in Fig. 7. These particles, therefore, have the potential to treat a variety of complex and resistant cancer cells, such as those originating in the colon, breast and pancreas [92].
Fig. (7). TRAIL/transferrin/DOX loaded HSA nanoparticles induce the destruction of cancer cells by synergistically acting through different mechanisms each. During clinical trials, these particles have shown favourable results against (i) HCT 116 cells: an apoptosis-working colon cancer cell-line, (ii) MCF-7/ADR cells: a resistant breast cancer cell-line, and (iii) CAPAN-1 cells: a necrosis- and apoptosis-less sensitive pancreas cell-line [92], reproduced by permission of Elsevier.

CONCLUSIONS

Extensive research for achieving therapeutic responses from anticancer medication, without inducing toxicity and drug resistance, resulted in the successful use of albumin as a drug carrier in tumour chemotherapy. Most chemotherapy treatment regimens are designed to provide site specific drug release, by increasing permeability and increased albumin transport through the endothelial blood capillaries of tumorous cells. Over years of research, several albumin-based drug delivery systems, such as conjugates, nanoparticles and microspheres have been developed and clinically evaluated. These anti-tumour agents have proven effective, because of their site specificity and fewer adverse effects, compared to conventional medication. An improved therapeutic index and safety with tumour therapy can therefore be achieved, by using albumin as a carrier for therapeutic agents in tumour management.
FUTURE PROSPECTS

From the studies reviewed here, it is plausible to say albumin, being a versatile biopolymer, has shown excellent cancer targeting and stands as an effective carrier for anti-tumour agents. However, this does not end here and there are more avenues yet to be explored. Most importantly, a better understanding of mechanisms of action of albumin-based delivery vehicles will provide a basis for their further optimisation and more improved drug delivery products could be foreseen. Albumin activity alongside its change in molecular weight before and after conjugation with the therapeutic entities is the potential area open for future exploration. Furthermore, sequential delivery of combination of chemotherapeutic agents, albumin as an anti-HIV agent, cationised albumin as a drug carrier to target blood-brain barrier will provide hope for new treatment options in the coming years after thorough physicochemical, pharmacological and immunological characterisation. In addition, several pipeline products based on albumin as discussed in this review are under developmental phase and are being scrutinised for long term and short term toxicity in animal and cell culture models before they can get the FDA approval for clinical trials. Nevertheless, with our continued thrive to mitigate and cure cancer, and with improved understanding of the molecular mechanisms involved in the cancer propagation provides us hope in developing more effective ways of treatment in the near future.

CONFLICT OF INTEREST

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.
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