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Review Article

CHITOSAN MICROSPHERES FOR THE DELIVERY OF CHEMOTHERAPEUTIC AGENTS: PACLITAXEL AS A MODEL

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ABSTRACT

Chitosan has unique physicochemical and biological features that suggest it as a good candidate for the development of safe and effective drug delivery systems. Linking drug molecules with chitosan through a functional spacer enables formulation of prodrugs that have appropriate pharmacological activities at specific desired sites. The development of formulations of targeted delivery systems for the chemotherapeutic agents, especially those with unfavorable pharmacokinetic features, like paclitaxel (PTX), can potentially alleviate the systemic cytotoxicity as well as directing therapy to the specific lesions. The main aim of this literature review is to critically evaluate the use of chitosan microspheres as a drug delivery system to enhance PTX distribution and efficacy in specific targeted sites.

Keywords: Chitosan, Paclitaxel, Microspheres, Drug delivery.

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INTRODUCTION

One of the most significant current diseases today is cancer. It is a leading cause of death worldwide [1]. Many strategies are committed to cure cancer including chemotherapy, surgery, and radiotherapy. However, these therapies have various types serious adverse effects such as hair loss, nausea, vomiting, fatigue, sleep problems, loss of appetite, and anemia [2,3]. During the clinical phase of the disease, most of the approved drugs failed to meet the targeted clinical responses, mostly due to inability to accumulate at the target site of action, where significant amounts distributed in normal tissues or organs rather than the diseased one; this will be accompanied with emergence of severe side effects [4]. In this regard, the most effective approach to avoid this problem is through utilizing the targeted drug delivery systems, where the active moieties were released and trapped within the targeted site of action. This approach may fulfill the criteria of patient compliance and increase the efficacy of the active agents through improving their pharmacokinetic properties [5,6]. In each drug delivery system, there are three major components: An active ingredient, targeting chromophore, and carrier system. The active drug could be incorporated either by passive absorption or chemical conjugation into the carrier system. Meanwhile, the choice of a proper carrier molecule represents a significant importance due to its influence on both the pharmacokinetic and pharmacodynamic properties of the active agent [7]. Novel polymeric drug delivery systems were incorporated as a novel approach for dispensing chemotherapeutic agents and have many advantages including availability of various biocompatible polymers that enables formulation of many pharmaceutical dosage forms that utilize polymer-based drug carriers, such as microspheres, hydrogels, micelles, and nanosystems [8,9]. The conventional formulations of chemotherapeutic agents have inherited critical drawbacks that include high toxicity rates due to indiscriminate distribution toward normal and diseased cells after systemic administration. Moreover, poor aqueous solubility of most anticancer agents needs to use organic solvents during their formulation, with associated rise of undesirable effects such as vascular irritation and respiratory distress [10,11]. Therefore, utilizing an effective carrier system that can be loaded with large amounts of the active drug and specifically targeting cancer cells is considered as an interesting idea for successful cancer treatment.

A wide range of chemotherapeutic agents has been formulated using the approach of loading in a carrier system for many reasons. Chitosan-loaded drugs represent the bases of delivering hydrophobic small molecular drugs to a specific site of action [12]; this type of delivery systems includes a water-soluble polymer conjugated to the active drug through a biodegradable spacer, which is stable at circulation while cleaved at the targeted cell spontaneously or by enzymatic catalysis. Based on this concept, many delivery systems are formulated and approved for clinical use. There are many examples for this system among them is gemcitabine-loaded chitosan microspheres, where the carrier system improves cytotoxicity of the active agent [13]. Similarly, nanoparticles of gemcitabine were prepared to utilize chitosan polymer, and evaluation of this formula showed that gemcitabine-loaded particles demonstrated optimum size and maximum entrapment properties [14]. Moreover, the recently prepared methotrexate (MTX)-loaded chitosan-based microspheres proved to be a promising drug delivery system that improves inject ability and compatibility, in addition to prolongation drug releasing time of injectable MTX [15]. Meanwhile, the magnetic chitosan-MTX microspheres prepared by suspension cross-linking technique demonstrated great potential application in magnetic targeting drug delivery technology [16]. Microspheres of chitosan cross-linked with polyethylene glycol were prepared by emulsion cross-linking and found to slow the release of 5-fluorouracil up to 24hr to colonic region and increased its anticancer activity [17]. Furthermore, various formulations of chitosan-based models loaded with doxorubicin and cisplatin were prepared and evaluated, and the results demonstrated promising features for these newly developed formulations to improve efficacy and safety of these cytotoxic agents [18-20].

At present, chitosan-based drug delivery systems are widely utilized to target specific sites within the gastrointestinal (GI) tract since this approach can protect the highly sensitive active moieties from the unfavored conditions of the upper GI compartment. This approach enables the release of the active agent at specific sites (e.g., the colon), probably through hydrolysis of the glycosidic linkage with chitosan by the microflora [21]. In this regard, among the most investigated chemotherapeutic agents is paclitaxel (PTX), and many evidence showed that cellular association and cytotoxicity of PTX were significantly enhanced when administered as chitosan-loaded formula [22]. However, despite its many clinical advantages, PTX suffers from many limitations including low bioavailability due to its poor solubility [23]. Recently, scientists have shown an increased interest in

formulating low therapeutic index drugs (e.g., PTX) in smart delivery systems, such as microspheres, to overcome such limitations and to avoid the fluctuations in plasma concentration of the drug, which lead to over or under medication [24]. Smart delivery systems are designed for the aim of providing safer and more efficient ways to deliver drugs with minimum adverse effects and/or enhanced therapeutic efficacy of the drug. These new drug delivery systems have become very important and might be more important than the drug itself. As a drug carrier, chitosan has been widely used as a drug delivery system for different types of biologically active molecules, including peptides, genes and small molecular weight drugs [25-27]. At present, many molecular targets are discovered and well characterized for therapeutic targeting, and the majority of clinically approved drugs belongs to the low molecular weight type [28]. Accordingly, effective delivery of these drugs, especially those with low therapeutic index or wide range of toxic effects, is considered as a growing challenge for researcher in pharmaceutical industry. The main aim of this literature review is to critically testing the use of chitosan microspheres as a drug delivery system to enhance PTX distribution and efficacy in specific targeted sites.

CHITOSAN MICROSPHERES

Microspheres are one of the most advanced approaches of controlled drug delivery systems [29]. They can be defined as spherical, small, biodegradable, and biocompatible particles [30] more than 1 μm (<100 μm) in size [31]. Due to the microsphere's small particle size, it has large surface area leading to a higher diffusion rate and better control of drug release [31]. Furthermore, there are several types of microspheres such as polymeric microspheres [32], radioactive microspheres [33], floating microspheres [34], and bioadhesive microspheres [35]. Microspheres have different applications in medical practice. In most instances, they are utilized as carriers and target delivery systems for many drug molecules, and for slow releasing of drugs over the required period to maintain effective drug concentrations at specific biological targets. In addition, microspheres have many other applications in food, medical devices, and water purification techniques [36]. Moreover, microspheres have many applications in pharmaceuticals manufacturing. They are used for the delivery of large molecules, fragile molecules such as proteins, vaccines, antibiotics, hormones, gene therapy, and anticancer drugs. The involvement of chitosan-loaded anticancer agents to formulate new drug delivery systems can lead to novel features of the nanoparticles, which might be explained partly due to the high surface area of the active ingredients delivery over the bulk properties [37].

Chitosan is as natural, mucoadhesive, nontoxic, biodegradable [38], and biocompatible polymer [39,40]. It is a product of chitin deacetylation and found in the exoskeleton of crustaceans. Due to chitosan's physical and chemical properties, its microspheres have many pharmaceutical applications [41,42]. Chitosan microspheres are used as a drug carrier to enhance the bioavailability of various drugs to get optimum drug release at specific site in the body. Furthermore, chitosan microspheres can be used in the formulation of either parenteral or oral drug delivery systems. Moreover, chitosan and its derivatives have the ability to be easily cross-linked with other molecules to synthesize fine particulates that can be utilized as effective drug carrier systems [43]. The process involves covalent binding of chitosan side chains with many functional cross-linking molecules, including glutaraldehyde and polyethylene glycol [44,45].

There are various advantages for the chitosan microspheres, including controlled drug release pattern that may be either constant or pulsatile [46], decrease the toxicity of drugs, overcome the problems associated with hydrophilic or hydrophobic drugs, and better patient compliance. This type of formulation can be used as injectable sustained drug delivery system and help the patients to avoid frequent doses [30]; it also improves drug absorption due to the increased *in vitro* adhesion [38]. Furthermore, chitosan microspheres are used to enhance drug delivery through the blood–brain barrier [47], where Hassan and Gallo in 1993 showed that the bioavailability of oxantrazole-loaded chitosan microspheres could be enhanced in the brain tissue [48]. On the other hand, microspheres have a few disadvantages. They are hard to maintain the effectiveness of the drug during the production process, it is difficult to produce large quantities and expensive to produce [30].

CHITOSAN MICROSPHERES FOR THE DRUG DELIVERY OF PTX

PTX is a natural and hydrophobic anticancer agent. It has been discovered by the National Cancer Institute in 1960 [1]. It is extracted from the bark of the pacific yew tree *Taxus brevifolia* [49]. PTX is an influential antitumor drug against ovarian cancer, breast cancer, head and neck cancer, lung cancer [50], leukemias, melanoma, and esophageal adenocarcinoma [51,52]. Although PTX is a highly cytotoxic drug, it has limited clinical applications because of low aqueous solubility (<1 μg/ml) [23]. The solubility problem is due to inherited physicochemical properties. PTX structure consists of a fused ring system and many hydrophobic substituents [53], and there are no functional ionizable groups. However, PTX is available as sterile non-aqueous solutions consist of Cremophore and ethanol [54]. Unfortunately, this formulation has been observed to induce many adverse effects such as hypersensitivity and neurotoxicity [55]. Due to the problems of poor solubility and adverse effects associated with the available PTX formulation, scientists are trying to formulate it in many alternative and effective drug delivery systems, such as cyclodextrin [56,57], dendrimers [58], nanoparticles [59], prodrugs [60,61], liposomes [62], 2-methacryloyloxyethl phosphorylcholine polymers [63], and microspheres [64,65]. Moreover, a study performed by Mita *et al*. involved a new drug delivery system devoid of cremophore and composed of porous PTX microspheres [66].

In addition to chitosan loaded PTX microspheres formulation, PTX was incorporated in many other forms of chitosan-based delivery system formulations. The technique of loading PTX on chitosan involves physical complex formation rather than change of the drug loading on the nanoparticles. This approach provides most selective and safe delivery system for PTX [67]. A thermos-gelling solution of chitosan-β-glycerophosphate salt and PTX was used as a local delivery system to prevent local recurrence of malignant tumors, without associated inflation and hyperthermia reported with parenteral formulation [68]. Furthermore, a hydrogel formula of PTX-loaded chitosan effectively decreases the number of CD34-positive vessels in subcutaneous 3LL tumors, indicating a strong inhibition of angiogenesis and tumor growth [69]. A PTX-loaded chitosan as thermos-sensitive hydrogel, which was modified by glutaraldehyde and polyvinyl alcohol for intratumoral delivery, was found as a promising approach to achieve effective sustained release and enhanced chemotherapeutic activity through in situ tumor injectable administration [70]. Recently, a new type of chitosan-based binary-copolymer systems containing PTX-loaded micelles and siRNA-loaded LDL could directly deliver siRNA and PTX to cancer cells, offering promising chances to overcome the problem of multidrug resistance in cancer cells [71]. Moreover, parenteral nanocomposite hydrogel containing PEGylated gold nanorods and PTX-loaded chitosan was developed to improve local tumor control and found to have superior effects on preventing tumor recurrence and improving survival in the HepS-bearing mice, compared with photo-thermal therapy alone [72]. It has been also shown that PTX incorporated into polymeric micelles based on tocopherol succinate-chitosan-polyethylene glycol-folic acid micelle is a potential drug delivery system of PTX for the effective treatment of the tumor and reduction of systemic toxicity, which may provide a useful alternative formula for intravenous administration of PTX [73]. Among the different studied drug carriers, microparticles particularly chitosan-based microparticles have been used to enhance the solubility of anticancer drugs such as oxantrazole [74], PTX [65], and MTX [75].

Microspheres have been approved to enhance the solubility, dissolution rate, and bioavailability of PTX [65,76]. Al-shdefat *et al*. demonstrated

that chitosan microspheres influence the dissolution of PTX [65]. The presence of PTX in porous, amorphous form and small particle size in the microspheres further enhances its dissolution. The high zeta potential values of the particles allow maintenance of their low particle sizes, which prevent aggregation of the microparticles and increase PTX dissolution rate. It has been previously reported that the medium molecular weight of chitosan formulae exhibited the highest PTX dissolution rate because of the highest aqueous perfusable microspheres [65,77], where 90% of the drug could be released from the nanosuspension.

In addition to enhancing the solubility and dissolution rate of PTX, chitosan microspheres have also been demonstrated to entrap high percentages of PTX. Kollipara *et al.* have measured the entrapment efficiency of PTX in chitosan microspheres utilizing high-performance liquid chromatography. Different formulas have demonstrated different entrapment efficiencies of PTX depending on the molecular weight of chitosan and pH of the formulation and it was between 21-83.7% [78]. Moderate acidic medium (pH 3-5) is the ideal cross-linking media to get the highest entrapment efficiencies of PTX (83%, 83.7%, and 73.7%) [65,79]. Moreover, it has been reported that the highest entrapment efficiencies of PTX when incorporated in poly(lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres were 81.55, 81.53, and 73.93%. Furthermore, the reported PTX entrapment efficiencies in microspheres were higher than the entrapment efficiencies in other types of carrier systems such as liposomes (61.02±1.61%) [62] and gelatin nanoparticles (25%) [80].

In this regard, many investigators have also studied the release pattern of PTX from chitosan microspheres. They found that PTX release pattern was rapid in the first hour; then, the cumulative release amount was increased within 24 hrs [65], and the drug release pattern varies between different microspheres formulae. They specifically considered the microspheres' particle size and the amorphous form of PTX as the two major factors that influence both dissolution and release pattern of PTX. Thus, the smaller particle sizes exhibited the highest drug release profiles. Moreover, the viscosity of chitosan could affect the release pattern of the drug with consequent impact on the biological activity [81,82]. It has been demonstrated also that increasing the film thickness of chitosan decreases the release pattern of a drug [83], in addition to the influence of the medium condition that affects the release pattern of PTX from microspheres [84]. Cho *et al.* studied the *in vitro* drug release pattern of PTX loaded chitosan microspheres in sodium salicylate as a medium and found that the solubility of PTX could be enhanced by 100 times in sodium salicylate without damaging the micellar structure of polymeric micelle drug delivery [84]. Although Al-shdefat *et al.* study involved PTX loaded chitosan microspheres and considered the only study in this regard, there are various studies which involve using other polymers with microspheres such as chitin microparticles [65,85], chitosan poly(lactic-co-glycolic acid) (PLGA) microparticles [22], PLA-PEG-PLA microspheres [79], PLA and ethylene vinyl acetate copolymer [63], and PTX-loaded PLGA microspheres containing isopropyl myristate [86].

CONCLUSION

This critical review has explained the delivery of PTX loaded chitosan microspheres. This drug delivery system has been designed to enhance PTX delivery at the tumor site and to reduce its side effects. The most significant finding in this review is that PTX limitations can be effectively avoided through incorporation in chitosan microspheres; thus, improving its solubility, dissolution rate, entrapment efficiency, and its release pattern. Accordingly, chitosan microspheres represent a promising future for the delivery of PTX.

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