CHAPTER 2

GENESIS OF WORK & OBJECTIVES

This chapter deals reviews the problems associated with synthesis of gold nanoparticles. The issues & challenges associated with delivery of anticancer drug, doxorubicin hydrochloride, & how nanoparticulate delivery system can provide effective solution to overcome the problems are highlighted. The objectives of the present investigation are described in this chapter which highlights our strategies to overcome the existing shortcomings.

GENESIS OF WORK AND OBJECTIVES

2.1 The status and strategy for delivery of doxorubicin hydrochloride

Cancer is one of the major causes of mortality worldwide and the incidence of cancer continues to rise. In 2007 alone, it claimed 7.9 million lives worldwide, which was 13% of all deaths.[1] In contrast to other diseases, cancer is a highly heterogeneous disease with diverse phenotypic expressions at different organ sites. Lack of effective diagnostic methods to detect cancer at its early onset, as well as a lack of efficacious therapy with minimal toxicity, remains the major limitation for complete eradication of the disease.[2]

The most widely studied anticancer drug till date is doxorubicin hydrochloride. It is an anthracyeline antibiotic, which intercalates DNA. It has been used in the treatment of a wide variety of cancers, such as acute lymphoblastic leukemia, multiple myeloma, carcinomas of the head and neck, breast, pancreas, stomach, liver, ovary, lung, prostate, uterus and neuroblastomas.[3] Despite being widely used, when doxorubicin is administered directly, lacks tumor targeting ability leading to narrow therapeutic effect and also its clinical use is hampered due to several undesirable side effects such as cardiotoxicity and myelosuppression.[4] Moreover, as doxorubicin is a hydrophilic molecule, restricted transport is observed through the cellular membrane leading to minimal drug internalization and its ability to overcome biological barriers, such as the blood brain barrier, is rather negligible. Due to this fact, free doxorubicin cannot successfully be used for the treatment of brain tumors.

Over the past several decades, with great development in the field of nanotechnology, various nanoparticles in different structures, shapes and composites provides good potential for their applications in cancer diagnostics and therapy. These nanoplatforms include polymer-drug conjugates, liposomes, polymeric micelles, polymersomes, etc.I5,6! In this regard, doxorubicin also has been exclusively incorporated into a number of nanocomposite platforms such as polymer-drug conjugates,[7,8] liposomes[9] and polymeric micelles.[10,11] Among all the nanomedicine platforms, liposomes have demonstrated the most clinical success with several FDA-approved formulations for cancer treatment.[6,12] A representative example is Doxil® (Ortho Biotech), a Poly(ethylene glycol)-liposome containing the drug doxorubicin. Doxil® was originally approved for the treatment of AIDS-related Kaposi's sarcoma and is now approved for use in ovarian cancer and multiple myeloma.[13] It circulates in the body as nanoparticles and has a half life –100 times longer than free doxorubicin. Its advantages over free doxorubicin are greater efficacy and lower cardiotoxicity. These advantages are attributed to passive targeting of tumours due to leaky tumour vasculature[14] and to lower concentrations of free doxorubicin at healthy

tissue sites. However, such nanoscaled systems have also shown that unwanted attributes can manifest themselves. Undoubtedly, pegylated doxorubicin liposomes have demonstrated a significant pharmacological efficacy in the treatment of Kaposi's sarcoma. However the polyethylene glycol coating results in preferential concentration of doxorubicin in the skin, which results in a side effect called palmar plantar erythrodysesthesia (PPE), more commonly known as hand-foot syndrome that is not observed with free doxorubicin.[15]

By design, a particle delivery system is required that is capable of sequestering a cancer drug solely within a tumor thus reducing the accumulation of the drug in healthy cells. While many approaches are being developed for nanotechnology enabled cancer diagnostics and therapeutics, a remarkably promising strategy involves the combination of noble metal nanoparticles especially gold nanoparticles and anticancer drugs. The unique chemical and physical properties of gold nanoparticles make them well suited for transporting and unloading the pharmaceuticals. The gold core is inert, non-toxic and biocompatible, making it an ideal starting point for carrier construction.[16] A second advantage is their ease of synthesis; monodisperse nanoparticles can be formed with core sizes ranging from 2 nm to 150 nm, whereas polymer based carriers can be heterogeneous in structure due to high polydispersity. For biomedical applications, the versatility is imparted by their ready functionalization with various ligands which are essential in order to target them to specific disease areas and allow them to selectively interact with cells or biomolecules. Additionally, for systemic applications, long circulating nanoparticles are desired for targeting the tumors sites. Functionalized gold nanoparticles with an outer Poly(ethylene glycol) shell can offer excellent stability in physiological conditions because Poly(ethylene glycol) has excellent solubility in water. The Poly(ethylene glycol) shell can greatly minimize the uptake of the gold nanoparticles by the reticuloendothelial system (RES), which can lead to a much longer circulation time in the bloodstream. This will allow the gold nanoparticles to preferentially accumulate in the tumor sites through the leaky tumor vasculature and provide flexibility to the attached ligand for efficient interaction with its target.[17] Although various methods for synthesis of gold nanoparticles have been reported, the most common synthesis route in aqueous solution involves Turkevitch process of reduction of gold (III) Cl_3 with trisodium citrate.[18] The two-phase Brust-Schiffrin method usually involves use of organic solvents,[19] however, the organic solvents render them unsuitable for biological application. Although numerous synthesis methods have been employed to fabricate gold nanoparticles, lack of sufficient stability has impeded the applications of gold nanoparticles. It is necessary to have a system which is water soluble, aggregation resistant, and biocompatible, does not include toxic chemicals in the synthesis protocol and introduce functionality to the particle surface for the conjugation of biomolecules for medical applications.

The prevalent methods for synthesis lack one or the other aforementioned properties. With this background the principal objectives of the present study comprised of the following:

1. To explore a natural, biocompatible polysaccharide gellan gum as reducing and stabilizing agent for the synthesis of gold nanoparticles.
2. To study the cellular uptake and in vivo oral toxicity of gellan gum reduced gold nanoparticles.
3. To obtain doxorubicin hydrochloride loaded gellan gum reduced gold nanoparticles and to evaluate its cytotoxicity against human glioma cell lines.
4. To study the functionalization of gellan gum reduced gold nanoparticles with biosurfactant sophorolipid and Poly(ethylene glycol).
5. To study the cytotoxicity of sophorolipid and Poly(ethylene glycol)-sophorolipid conjugated gold nanoparticles and their doxorubicin hydrochloride loaded derivatives towards human glioma and human glioma stem cell lines.
6. In-vivo tumor microvessel density evaluation-Tumor angiogenesis inhibition study

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