Physica Medica 48 (2018) 76–83

Contents lists available at ScienceDirect

Physica Medica

journal homepage: www.elsevier.com/locate/ejmp

Original paper

Multifunctional Chitosan-Capped Gold Nanoparticles for enhanced cancer chemo-radiotherapy: An invitro study

Mohamed Mahmoud Fathy[b,*,1], Fayrouz S. Mohamed[b,1], NihalSaad Elbialy[a,b,1], Wael M. Elshemey[b,1]

[a] Physics Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia
[b] Biophysics Department, Faculty of Science, Cairo University, 12613 Giza, Egypt

ARTICLE INFO

Keywords:
Gold nanoparticles
Chemo-radiotherapy
Doxorubicin
Radiosensitizers
Breast cancer
Chitosan

ABSTRACT

Over the last decade, chemo-radiotherapy represented a well-established paradigm for cancer treatment. Developing new strategies to promote the therapeutic efficacy while reducing toxic side effects of chemo-radiotherapy is a main research objective in cancer therapy. A promising new oncological strategy for enhancing chemo-radiotherapy against cancer involves the utilization of multifunctional nanoparticles (nanocarriers and radiosensitizers). In this work, Chitosan-Capped Gold Nanoparticles (CS-GNPs) were synthesized and loaded with an anticancer agent, Doxorubicin (CS-GNPs-DOX). The prepared multifunctional nano-formulation acted as nano-radiosensitizer, in addition to being an intrinsic drug delivery system allowing efficient loading and targeting of chemotherapeutics. The therapeutic efficacy of CS-GNPs-DOX was studied by treating breast cancer cells (MCF-7) with CS-GNPs-DOX accompanied by different doses of X-rays (0.5, 1 and 3 Gy) and assessing the cytotoxic effect via neutral red cell viability assay. Further assessment of the therapeutic efficacy was conducted using flowcytometry to measure the induction of apoptosis, while neutral comet assay was carried out to check DNA double strand breaks. Results showed that CS-GNPs-DOX could enhance the chemo-radiotherapeutic effect by significantly decreasing cancer cells viability with increasing DNA double strand breaks and inducing cell necrosis even at a very low radiation dose (0.5 Gy). Interestingly, the developed multifunctional CS-GNPs-DOX provided a synergistic regimen for cancer treatment that effectively delivered DOX to tumor cells and enhanced the radiosensitization activity, thus minimizing conventional radio-therapeutic required doses. Accordingly, CS-GNPs-DOX represents a promising multifunctional nanoparticle for enhancing breast cancer chemo-radiotherapy.

1. Introduction

Breast cancer is a major cause of cancer mortality and morbidity worldwide and is the most common cancer in women [1]. It has been ranked as the second cause of cancer death in women in the developed countries and the fifth cause of cancer death worldwide [2]. Local surgery, chemotherapy, radiation therapy, hormonal therapy and/or combination therapy are the major cancer treatment approaches [3].

Chemotherapy is limited by severe toxic side effects and reduced efficacy. This is partly owing to lack of tumor targeting (in the form of nonspecific distribution of drugs in body organs), low tumor retention and insufficient tumor deposition and penetration which are necessary to achieve cell killing. On the other hand, some chemotherapeutic drugs are hydrophobic and poorly soluble, thus their therapeutic efficacy are hindered. Moreover, resistance to chemotherapeutics is promoted upon repeated treatments with the same drugs [4,5].

In spite of the huge advances in treatment planning and dose delivery techniques, radiotherapy still suffers from the inability to completely avoid undesirable damage to normal healthy tissues surrounding the tumor after exposure of tumor to high therapeutic doses of ionizing radiation. The low radiation absorption efficiency of tumors and tumor radio-resistance, mainly due to the presence of hypoxic tumor cells, limit the efficacy of conventional radiotherapy [1,6–8].

Concomitant use of chemotherapy and radiotherapy (chemo-radiotherapy) has emerged as an important treatment paradigm in the curative management of more than 50% of all cancer patients [9]. In chemo-radiotherapy, the administered chemotherapeutics regulate the systemic metastasis, in addition to acting synergistically with local radiotherapy to control the primary tumor [10]. Large number of randomized clinical trials has shown that chemo-radiotherapy can solve

* Corresponding author.
E-mail address: mfathy@sci.cu.edu.eg (M.M. Fathy).
[1] This manuscript was written with contributions from all authors.

https://doi.org/10.1016/j.ejmp.2018.04.002
Received 16 November 2017; Received in revised form 2 April 2018; Accepted 4 April 2018

the problems of sub-lethal damage repair and hypoxic-related radioresistance compared to sole administration of chemotherapy or radiotherapy [11,12]. Despite these advantages, concurrent use of both chemotherapy and radiotherapy largely increases the induced toxicity to normal tissues which results in more severe systemic damage compared to the use of a single treatment alone. This is a major limitation on the clinical use of the combination therapy [13,14].

Within this context, several new approaches have been developed in order to improve the therapeutic index of chemo-radiotherapy. These generally involve the incorporation of molecularly targeted nanocarriers so as to increase the local effect in the tumor while minimizing the damage to normal tissue.

One strategy to enhance the clinical outcome of chemo-radiotherapy involves the use nanotechnology. Multifunctional nanoparticles (the effective units of nanotechnology) offer superior features that provide preferential delivery of chemotherapeutics to tumor sites as well as increasing local radio-therapeutic dose [15,16]. Recently, various nano-radiosensitizers have been investigated [17,18], among which substantial interest is directed towards Gold Nanoparticles (GNPs) [19–21]. As an element of high Z number (Z = 79), GNPs are known to significantly absorb more radiation (for instance, X-rays) than tissue [22,23]. Therefore, GNPs accumulated at the tumor site can concentrate radiation and give better dose conformation to tumor while minimizing normal tissue damage [24]. X-rays interact with GNPs through different mechanisms based on the energy of incident photons. In the kV energy range, the photoelectric effect is the dominant mechanism. In clinical radiotherapy, the Compton Effect is the most common interaction at the energies levels 6–20 MV [25]. The photoelectric mechanism results in production of low-energy electrons (Auger electrons) that have a range of few micrometers causing highly localized ionizations when released inside a tumor. This in turn results in enhanced radiobiological dose conformation [26–28]. The highest possible enhancement factor with GNPs can be achieved at the energy range of 40–50 keV, where the ratio of mass energy absorption coefficients of gold to water is maximum. Unlike the MV radiation, the use of kV energies is, unfortunately, limited by its shorter penetration and higher dose to skin. One may expect that at MV energies, no significant radiosensitization is expected as the ratio of mass energy absorption coefficients of gold to water becomes theoretically negligible and the contribution of the photoelectric-dependent dose enhancement is reduced. Surprisingly, the enhancement effect has been also reported at MV energies. One possible reason is that at higher energies, photon interaction with matter produces Compton electrons with an energy spectrum that have higher absorption coefficient with GNPs compared to the surrounding biological matter [29,30]. This is supported by Monte Carlo simulations reporting an increase in secondary electrons production when gold is irradiated with X-rays at 6 MV compared to water [31]. Moreover, it has been reported that some chemical and biological mechanisms may also help in boosting the radiosensitization effect of GNPs at MV energies [32]. The possible chemical mechanisms of GNPs radiosensitization include sensitization of DNA to radiation-induced damage and increased free radicals formation, while the biological mechanisms involve the interactions of the GNPs with the biological target molecules. These interactions induce some modifications to the pathways involved in DNA repair, cell cycle checkpoints and progression as well as cell proliferation, which results in achieving higher level of cell killing by radiation [26,30].

Although several new combined oncological approaches have been developed, studies concerned with the introduction of GNPs in chemo-radiotherapy are considerably limited.

Owing to the instability of the uncoated GNPs and their high susceptibility to form aggregates in physiological environments, several stabilizing and coating agents have been utilized in the synthesis of stable and biocompatible GNPs. The ability of some naturally occurring biodegradable polymers to produce stable GNPs has been studied [33,34]. Among them, chitosan is attracting much more attention due to its favorable pharmaceutical characteristics such as biocompatibility, biodegradability, susceptibility to chemical modifications and controlling drug release [35].

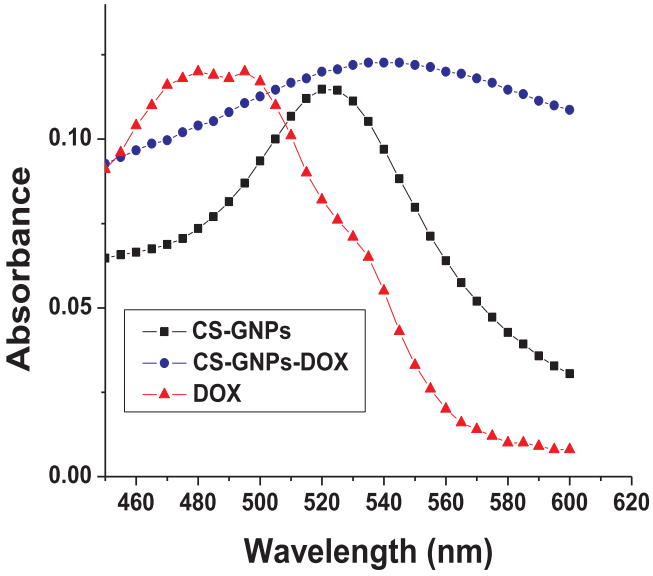

Fig. 1. The absorption spectra of CS-GNPs, CS-GNPs-DOX and free DOX.

Inspired by the structure of chitosan to enable efficient chemotherapeutic (for instance, DOX) loading, as well as the capability of gold (Au) as a high-Z element to act as a radiosensitizers, herein, Chitosan-Capped Gold Nanoparticles loaded Doxorubicin (CS-GNPs-DOX) are introduced as multifunctional nanoparticles for enhancing cancer chemo-radiotherapy. Where DOX, as abroad-spectrum anticancer drug, would act as a chemotherapeutic agent while CS-GNPs would sensitize tumor cells to radiation.

2. Materials and methods

2.1. Materials

Gold (III) chloride ($HAuCl_4{\cdot}3H_2O$, 99.99%), high molecular weight chitosan, doxorubicin hydrochloride (DOX), neutral red kit, trypsin, sodium chloride, sodium citrate, EDTA tetrasodium salt, ethidium bromide dye, triton X-100, sodium lauroylsarcosine, phosphate-buffered saline (PBS) and tris buffer were purchased from Sigma Aldrich. Absolute ethanol and hydrochloric acid (HCl) were purchased from Merck. MCF-7 cell line was obtained from the American Type Culture Collection (ATCC). Fetal bovine serum, Normal Melting and Low Melting Point Agarose (NMPA, LMPA, respectively) were bought from Gibco. Dulbecco's Modified Eagle Medium (DMEM), L-glutamine and penicillin–streptomycin-solution were purchased from Lonza. FITC Annexin V Apoptosis Detection Kit was purchased from BD Biosciences. Deionized water was used throughout the study.

2.2. Methods

2.2.1. Synthesis of Chitosan-Capped Gold Nanoparticles loaded with Doxorubicin (CS-GNPs-DOX)

100 ml of 0.05% chitosan solution prepared in 1% acetic acid was heated while stirring till boiling. Then, 200 µl of tetra-chloroauric acid aqueous solution (49 mg of $HAuCl_4{\cdot}3H_2O$/ml deionized water) was added. The color of reaction mixture was converted to the characteristic ruby red indicating the formation of gold nanoparticles. During rinsing, the sample was separated from the supernatant using centrifugation (14,000 rpm, for 30 min) to remove the excess chitosan. The gold concentration was calculated from the total weight of gold (4.9 mg)