ORIGINAL RESEARCH PAPER

Medical Physics

THERAPEUTIC POTENTIAL OF CURCUMIN NANOPARTICLES AGAINST HUMAN COLON CANCER CELLS (HCT116)

KEY WORDS: Curcumin, Mesoporous silica nanoparticles, Cancer, Drug delivery, Colon cancer cell line (HCT116).

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ABSTRACT

Curcumin, a magical molecule derived from the rhizome of Curcuma longa has gained immense importance for its potent anticancer activity; however, the therapeutic potential is restricted by low bioavailability. The study aims to provide a potential drug nanocarrier based on curcumin (CRM) loaded mesoporous silica nanoparticles (NPs) and coated with poly glycol Ethylene (NPs-CRM), which targets the cancer cells without destroying the normal cells. The physico-chemical properties of NPs-CRM were characterized using transmission electron microscope (TEM). NPs-CRM was displayed high loading amount of curcumin in the pores of NPs. In vitro studies exhibited that NPs-CRM induces higher cytotoxicity in colon human cell line (HCT116) related to CRM alone. The results revealed that mesoporous silica nanoparticles contribute in improvement the poor bioavailability and solubility of curcumin considerably. In summary, this study adds a novel and an efficient nanodrug delivery system enhancing the therapeutic efficacy of curcumin significantly.

INTRODUCTION

Cancer is a dreadful disease that prevalent in many parts of the world. It has become the most common leading cause of death worldwide[1]. Chemotherapy, radiotherapy, and surgery are the most common conventional approaches used for cancer treatment in a combination of them or alone. In spite of the approaches have high efficacy in killing the cancer cells, but lack the ability to discriminate between cancer and normal cells[2]. Therefore, it is important to identify save alternative approaches that have anticancer properties such as using plants-derived bioactive natural products as nutraceuticals.

Curcumin is a hydrophobic natural polyphenol which is extracted from the roots of perennial plant Curcuma longa. It is the active ingredient of the famous flavoring and coloring spice in Asia, turmeric[3]. A wide range of studies have confirmed that curcumin can exhibit several pharmacological properties involving anti-inflammatory[4], antioxidant[5], antimicrobial[6], antibacterial[7], and anticancer[8]. In addition, studies demonstrated that this compound has potential therapeutic effects against several diseases such as cancer [8], Alzheimer disease[9], cardiovascular disease[10], and autoimmune disease[11]. Moreover, curcumin as anticancer agent has been shown to induce apoptosis, inhibit cell proliferation, and suppress carcinogenesis in several cancer cells[12]. Although promising pharmacological and therapeutic activities of curcumin, it has not been approved by FDA as a drug and its clinical uses still restricted by low bioavailability, poor absorption, and rapid metabolism[13]. To overcome these obstacles, several drug delivery systems using nanoparticles, nanocarriers, have been investigated.

Nanocarriers are a promising novel strategy which used for enhancement the delivery of natural anticancer drug curcumin by its loading or encapsulating inside them. Several types of nanoparticles can be used for designing the nanocarriers such as: solid lipid nanoparticles[14], micelles[15], gold nanoparticles [16], silver nanoparticles[17], liposomes[18], niosomes[19], and mesoporous silica nanoparticles[20].

Mesoporous silica nanoparticles have gained considerable attention for various properties as drug carrier. They exhibit good chemical stability, high surface area and pore volume, and tunable pore size that make them a promising candidate for enhancement the delivery of many low bioavailable drugs, particularly related to cancer therapy[21].

Herein, we developed an efficient nanocarrier of mesoporous silica nanoparticles for loading the hydrophobic drug, curcumin, which coated with poly ethylene glycol (PEG) resulting (NPs-CRM) to study the influence of CRM loaded on the mesoporous silica nanoparticles compared to curcumin alone (CRM) on human colon cancer cell line HCT116 and evaluate their therapeutic efficacy as anti-cancer agents.

METHODOLOGY:

Mesoporous silica nanoparticles (NPs) were purchased from Sigma-Aldrich (USA). Then, the purchased nanoparticles were characterized using transmission electron microscopy TEM (FEI, HR-TEM, Netherland), to confirm the synthesis and morphology of NPs. Mesoporous silica nanoparticles (NPs) were loaded with curcumin (CRM) [Sigma-Aldrich, USA] and then coated with PEG creating (NPs-CRM). The encapsulation efficiency of NPs was calculated. After that, colon cancer cell line (HCT116, ATCC, USA) was obtained from Stem Cells Unit at King Fahad Center for Medical Research (KFMRC), grown in 96-well plates with recommended Dulbecco's modified medium (DMEM) media (Gibco, USA), and incubated for 24 h at 37 in 5 % CO_2 atmosphere. Then, NPs-CRM and an equivalent dose of curcumin alone (CRM) were added to each plate and incubated for another 24 h. After that, 10 µl of Cell Proliferation assay kit WST (ABCAM, UK) was added to each well and incubated for 4h. Then, the cytotoxicity effect of CRM alone and NPs-CRM on HCT116 was assessed. The morphological changes in HCT116 by CRM and NPs-CRM were analyzed using light inverted microscope (OLYMPUS, inverted system microscope, Japan) after and before treatment (24 h incubation).

STATISTICAL ANALYSIS: All data were presented as mean ± standard division SD, which is represented by error bars using Origin 8.

RESULTS AND DISCUSSION: TEM images of mesoporous silica nanoparticles (Figure 1 a & b) revealed the spherical shape of the NPs with smooth surface. Furthermore, the images showed presence of channels-like pores on the surface of NPs. The encapsulation efficiency after loading of curcumin was achieved satisfactory results which was about 92.4 %.

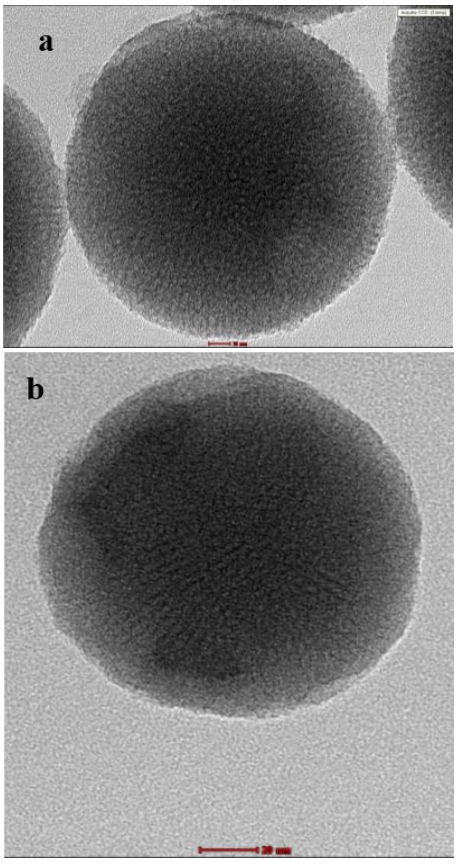

Figure (1): TEM images of mesoporous silica nanoparticles (a) and zoomed image of one nanoparticle (b).

The cytotoxicity effect of CRM alone and NPs-CRM was investigated using WST-1 assay on HCT 116 after 24h (BioTek, Elisa reader, USA) and then the results were further confirmed using inverted electron microscope (OLYMPUS, inverted system microscope, Japan). After 24 h of drugs incubation, the cytotoxicity of the two drugs on HCT116 was evaluated with different concentrations (108, 90, 72, 54, 36, & 18 µg/ml). CRM alone exhibited very low cytotoxicity at all different concentrations where the higher dose (108 µg/ml) of CRM showed mild toxic effect (the cell viability was about 65.9 ± 3.3 %) as shown in Figure (2). Meanwhile, Nps-CRM decreased the survival rate significantly Figure (2). The cell viability related to the higher dose of NPs-CRM was about 10.6 ± 0.53 %.

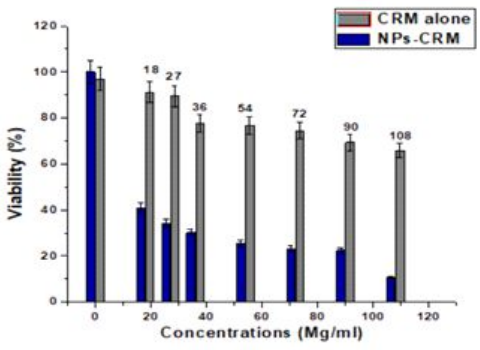

Figure 2: Viability of HCT 116 cells incubated with CRM alone and NPs-CRM for 24h

Consequently, as depicted in Figure (2), NPs-CRM suppressed the proliferation rate of HCT116 cells significantly in a dose dependent manner when compared to those control and cells treated with CRM alone. However, CRM alone did not show significant cytotoxic effect on HCT116 as compared to cells treated with NPs-CRM.

The morphological shape of both untreated cells and treated with CRM and NPs-CRM was evaluated after 24 h using inverted light microscope as showed in Figure (3, 4, & 5) for more confirmation of the cytotoxicity results.

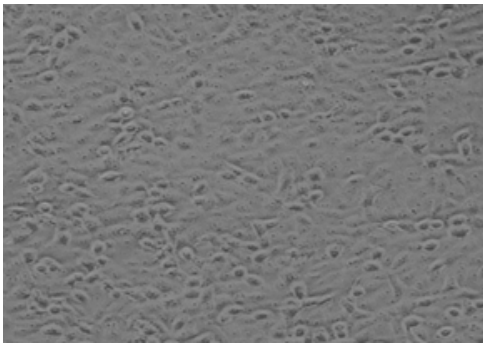

Figure 3: Inverted Electron microscopy image of HCT 116 cells, untreated (control) cells (20 x).

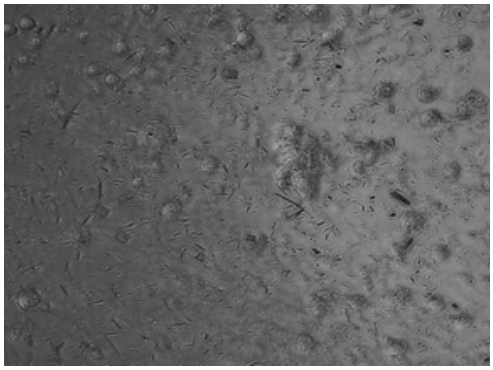

Figure 4: Inverted Electron microscopy image of HCT 116 cells incubated with CRM alone for 24h (40 x).

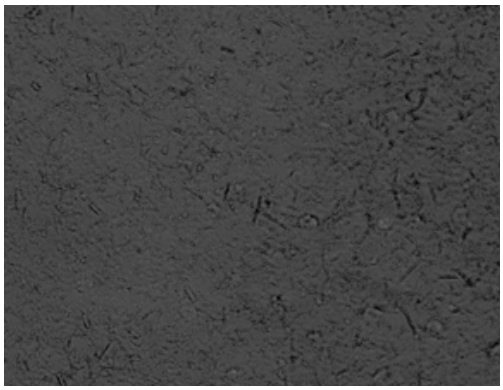

Figure 5: Inverted Electron microscopy image of HCT 116 cells incubated with NPs-CRM for 24h (40 x).

The untreated cells (control) showed the epithelial and spindle shape of HCT116 Figure (3). CRM-treated cells were exhibited very little apoptosis with change in their shape compared to control cells as well as low cytotoxicity effect as shown in Figure (4). This result is consistent with previous study[22]. This can be attributed to the fast clearance and degradation of curcumin from the cancerous cells[23]. However, Figure (5), cells treated with NPs-CRM were displayed higher apoptosis which accompanied with great change in their shape compared to those untreated and treated with CRM alone. Additionally, they showed high levels of

cytotoxicity as shown in Figure (2). Interestingly, the Light microscopic images demonstrated that most of HCT116 cells post treatment with NPs-CRM appeared in rounded shape and loss their contact, some of them became floating, and another suffered shrinkage and distortion in their shape. Additionally, the images showed some black aggregates[24] in CRM-NPs-treated cells indicating the accumulation of curcumin inside them as depicted in Figure (5). These results demonstrated that NPs-CRM have the potential to inhibit the tumor growth significantly compared to CRM alone

Furthermore, NPs-CRM exhibited high levels of cytotoxicity against HCT116 cells related to CRM alone. This reveled the high efficacy of curcumin loaded to mesoporous silica nanoparticles against cancer cells as anticancer agent. In addition to, mesoporous silica nanoparticles enhance curcumin bioavailability considerably. Furthermore, Coating MSNPs-Cur with PEG protects curcumin molecules to be degraded or released under physiological condition (pH = 7.4) while stimulates the release of entrapped curcumin in the acidic environment (pH = 5.5)[23,25,26].

CONCLUSIONS

To conclude, we have developed a nanocarrier system utilizing the large surface area and pores properties of mesoporous silica nanoparticles to load the promising anticancer drug, curcumin. Furthermore, NPs have save use as organic material. NPs-CRM showed a considerable drug sustained release and higher apoptosis at tumor microenvironment. Additionally, NPs-CRM exhibited significant cytotoxic effects in time and dose dependent way in comparison with CRM alone-treated cells. Our findings indicated that curcumin loaded NPs improved significantly curcumin bioavailability as well as antitumor potential. These results propose that NPs-CRM might be a novel alternative anticancer to that conventional cancer therapies for transporting the hydrophobic anticancer drugs to cancer cells. More experiments on another cell lines and in vivo experiments is under investigation in our laboratories. More innovative methods for utilizing the properties of mesoporous silica nanoparticles and curcumin as anticancer agent are needed to transport them from lab to clinic.

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