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Photodynamic therapy of cancer cells using folate conjugated gold nanoparticles as a new nanoplatfrom for targeted cancer therapy

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Abstract---Photodynamic therapy works through photo activation of a specific photosensitizer in a tumor in the presence of oxygen. PDT is widely applied in oncology to treat various cancers as it has a minimally invasive procedure and high selectivity, does not interfere with other treatments, and can be repeated as needed. A large amount of reactive oxygen species (ROS) and singlet oxygen is generated in a cancer cell during PDT, which destroys the tumor effectively. In this study, explained utilization of gold nanoparticles as a photosensitizer in photodynamic therapy of primary liver cancer and colorectal cancer that showed gold NPs exerted cytotoxic effects on cancer cells in dose dependent manner. In this study also used folic acid (a water-soluble vitamin) as ligand to increase targeting of nanoparticles to cancer cells were tumor cells over-expressing folate receptors for their rapid proliferation. The results showed that the folic acid conjugated Au NPs exerted a significant cytotoxic effect on cancer cells (decrease in cells viability) ($p < 0.001$) and upon irradiations the effects also enhanced significantly comparing with gold nanoparticles alone.

Keywords---photodynamic therapy, primary liver cancer, colon cancer, gold nanoparticles.

Introduction

Cancer

It is commonly accepted that cancer is an important public health problem with a high mortality throughout the world, primary liver cancer is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer mortality worldwide as hepatocellular carcinoma (HCC) generally occurs in the presence of liver disease or cirrhosis(Ding, Tu et al. 2021), and at least 60% of HCC cases worldwide are caused by viral hepatitis(de Martel, Maucort-Boulch et al. 2015). Chronic hepatitis B virus (HBV), typically acquired at birth or early childhood, remains the leading cause in most high-risk HCC areas. Colorectal cancer is the fourth most commonly diagnosed cancer. stages of colon cancer include four stages according to its spread and invasiveness(Engstrom, Arnoletti et al. 2009). In spite of the broad used of conventional chemotherapeutic agents for treating cancer, these agents have some disadvantages, including inadequate tumor tissues recognition leading to damage to the healthy tissues and low bio-compatibility caused by their hydrophobicity, which results in the aggregation(Bray, Ferlay et al. 2018, Dag, Omurtag Ozgen et al. 2019) .To overcome these disadvantages photodynamic therapy are being used in cancer therapy(Gomes, Martins et al. 2021).

Photodynamic Therapy

Photodynamic therapy is a type of treatment approach suitable for specific types of conditions which are not cured by surgical resection and include various cancer types. PDT is considered as a promising treatment modality, presenting significant effectiveness and limited side effects, while numerous side effects are associated with conventional chemotherapy. In PDT, a non-toxic agent known as photosensitizer (PS) is administered and taken up by tumor cells, before being activated by light of a specific wavelength that correspond its absorption properties. Then, light-activated PS induces selective damage to tumors and surrounding vasculature in the presence of molecular oxygen(Calixto, Bernegossi et al. 2016).

Gold nanoparticles

Gold NPs are the most well-suited nanomaterial, surface Plasmon resonance property makes them useful nanomaterial in different fields like bio imaging, biomedical therapeutics and bio diagnostic tools. Biomolecule Au NPs are used in the medicine and in cosmetic products. These are also used as catalyst and for novel coatings and paintings. (Dhanalekshmi, Sangeetha et al. 2020). Gold NPs consider the most important laser-responsive nanomaterial, into the tissue changes optical properties of the medium and increases local conversion of laser energy into heat. AuNPs possess surface plasmon resonance (SPR), a well-known property that implies the resonant oscillation of free electrons on the particle surface induced by incident light (Raoof, Cisneros et al. 2013).In order to limit such released heat and its resulted thermal damages to the tumor, and also to increase targeting of nanoparticles towarded tumor cells it is necessary to target AuNPs towards cancer cells using various targeting ligands such as folate. The

combination of F-Au NPs with laser exposure leads to selectively heat and destroy the cancer cells (Mehdizadeh, Pandesh et al. 2014).

Method and Materials

In this experimental study, PLC/PRF/5 and SW480 cell lines were treated with gold nanoparticles stock solutions in different concentrations (500, 250, 125, 62.5 and 31.25 $\mu\text{g/ml}$) over incubation periods of 24 hours. The present study was approved by Ethical Committee of college of medicine- University of Babylon.

Preparation of gold Nano particles (Turkevich method)

It's a chemical procedure where gold Nano particles are prepared as a solution, it's simple and result in aspherical gold nanoparticles which is testing by TEM (Transmission Electron Microscopy), (Wang and Dellago 2003)

Materials include

- A-Chloroauric acid
- B-Trisodium citrate dihydrate (TCD) as reducing agent.

For preparing the Gold Nano particles a stock solution was made from Chloroauric acid and trisodium citrate dihydrate, then to prepare this stock solution of 2% Chloroauric acid we dissolved 1g of Chloroauric acid, to prepare the Gold Nano particles 2% Chloroauric acid, and for preparing 1% stock solution of Trisodium citrate dihydrate dissolve 1g in 100ml deionized water. After preparing the stock solutions, 150 μl of Chloroauric acid solution plus 50ml of deionized water must heat up to 100°C then 500 μl of Trisodium citrate solution was added (stirrer with heating at 100°C) until the clear color change into red color indicating the gold Nano particles.

Folic acid stock solution preparation

Folic acid (pure drug powder) was solubilized with DMSO then 20 milligrams was dissolved in 1ml of DMSO to obtain a stock solution of the drug 20,000 $\mu\text{g/ml}$ and from this stock a serial dilution was made.

Laser Irradiation

The irradiation was done in a 96-well plate after 24 hours of cell seeding. The cells were uniformly irradiated and there was a distance of 10 centimeters between the laser tip and the bottom of the plate, which corresponded to a single well size from the 96-well plate (Dompe, Moncrieff et al. 2020). In this study used red laser with wave length 635 nm, laser power 300 Mw with continuous wave.

Cell Culture

The PLC cells were obtained from Tissue Culture Laboratory in the College of Medicine - University of Babylon. Cells were grown in RPMI-1640 with sodium bicarbonate powder according to need in addition to 10% fetal bovine serum (FBS)

and gentamycin (50 µg /ml). Sterilization was done by 0.4 and 0.2 µm Millipore filters subsequently. Cultures were incubated at 37°C. SW480 cell line was also obtained from Tissue Culture Laboratory in the College of Medicine - University of Babylon and also grown in RPMI medium

Cytotoxicity assays

MTT Assay

The general purpose of the MTT assay is to measure viable cells in relatively high throughput (96-well plates) without the need for elaborate cell counting.

Statistical analysis

Both standard deviation and Means are calculated in this experimental study. The differences between the data that are statistically significant of obtained from the experimental was analyzed via analysis using a one-sample test by SPSS (25 version) one-way Anova computer program. P-value <0.05 was considered significant while <0.001 was considered highly significant.

Results

Cytotoxicity evaluation of Folic acid on the viability of PLC and SW480 cancer cell line

The results of folic acid on PLCs liver cancer cell lines and SW480 colon cancer cell lines showed that all the concentrations statistically decrease in cell viability ($p < 0.001$) compared with the control.

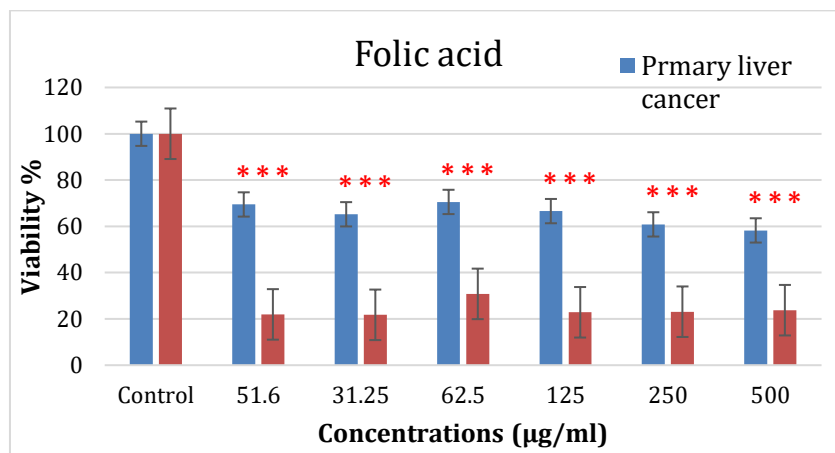


Figure 1. effect of Folic acid on viability of PLC and SW480 cancer cell line using MTT assay

Cytotoxicity evaluation of Gold NPs on the viability of PLC and SW480 cancer cell line

The results of Gold NPs on PLCs liver cancer cell lines showed that the concentrations Only 500 and 250 $\mu\text{g/ml}$ significantly decrease in cell viability ($P<0.050$) differ from the control. The results of Gold NPs on SW480 colon cancer cell lines showed that the Concentrations from 500-62 $\mu\text{g/ml}$ significantly decrease cell viability ($P<0.001$) differ from the control, while 31.5 $\mu\text{g/ml}$ differ ($P<0.050$) from the control.

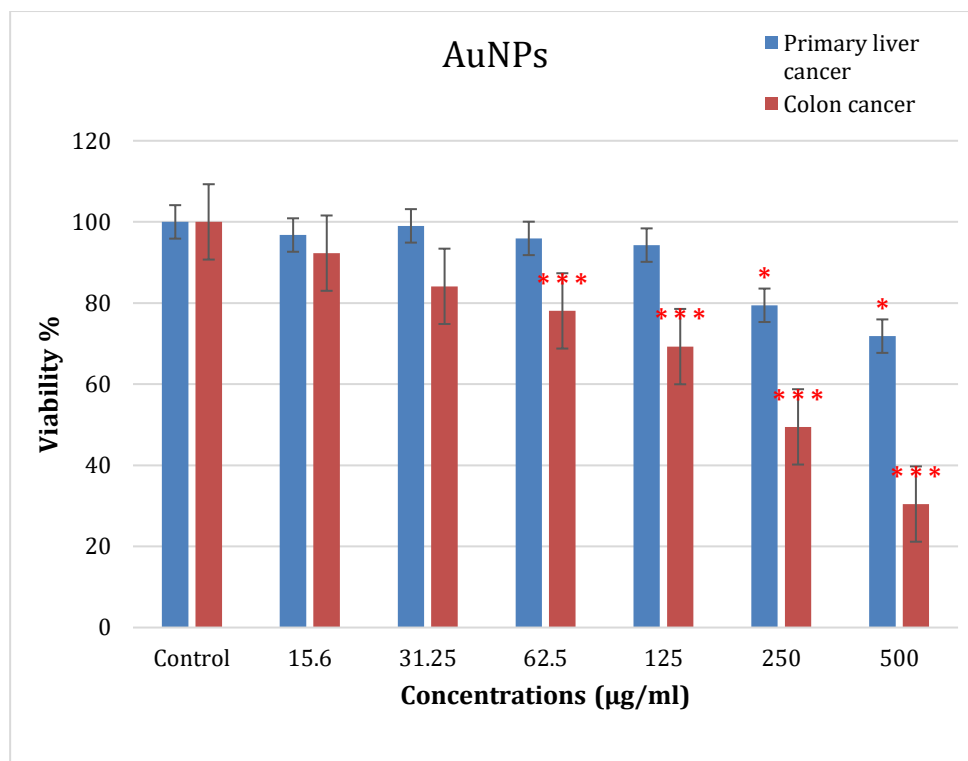


Figure 2. effect of Au NPs on viability of PLC and SW480 cancer cell line using MTT assay

Cytotoxicity evaluation of Folic acid combined Gold NPs on the viability of PLC and SW480 cancer cell line

The results of Gold NPs with folic acid on PLCs liver cancer and SW480 colon cancer cell lines showed that all the concentrations statistically decrease in cell viability ($p<0.001$) compared with the control.

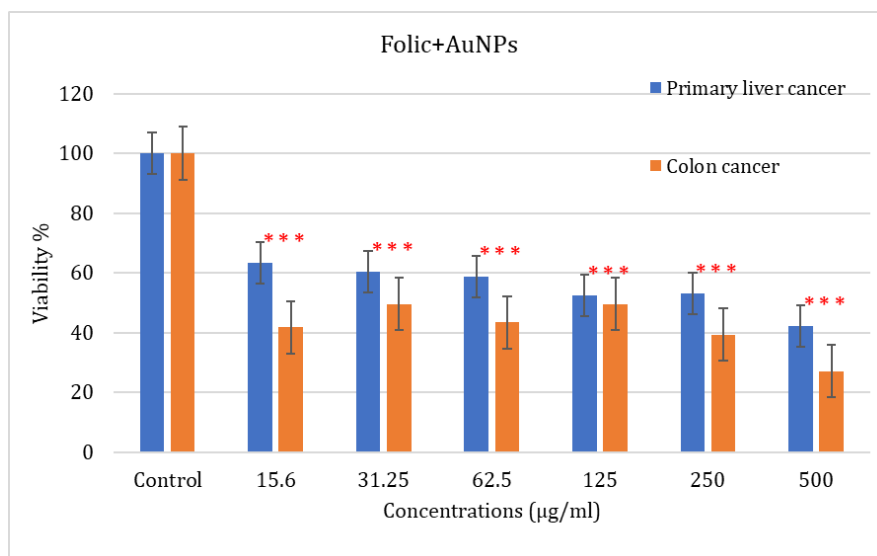


Figure 3. effect of Folic acid combined Au NPs on viability of PLC and SW480 cancer cell line using MTT assay

Cytotoxicity evaluation of Gold NPS combined with folic acid and laser on the viability of PLC and SW480 cancer cell line

The results of Gold NPs with folic acid and laser on PLCs liver cancer and SW480 colon cancer cell lines showed that all the concentrations statistically decrease in cell viability ($p < 0.001$) compared with the control.

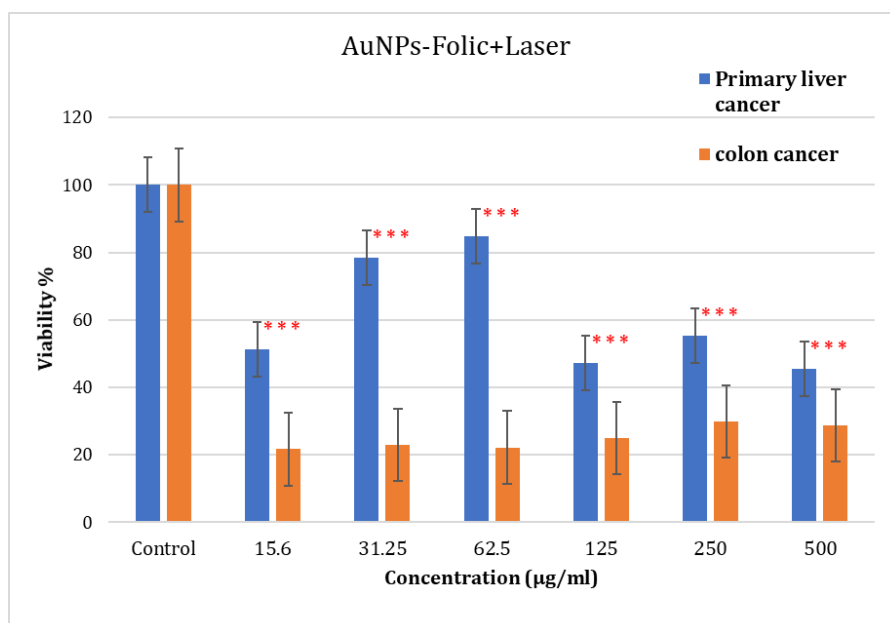


Figure 4. effect of Au NPS combined folic acid with laser on viability of PLC and SW480 cancer cell line using MTT assay

Discussion

Cytotoxic effect of folic acid on primary liver cancer and colon cancer cell lines

Folic acid is a small water-soluble molecule and vitamin in the B group. Folate plays an important role in regulating DNA replication and various metabolic pathways such as the biosynthesis of thymidylates and purines so a sufficient intake of folate is needed in any rapidly proliferating cells like cancer cells (Cheung, Bax et al. 2016). FA can protect against the development of cancer by helping in methionine regeneration and reducing the error rate during DNA replication. In addition, FA has been shown to exert the scavenging potential for free radical and antioxidant properties, which might also contribute to its anti-tumor properties (Li, Rong et al. 2006). The results of this study showed that there is a statistically significant decrease in cell viability percent ($p < 0.001$) at all concentrations for both cell lines (PLCs and SW480) this results closed to this study that indicated FA inhibit cell proliferation by inhibits tyrosine kinase activity particularly epidermal growth factor receptor (EGFR) suggests a role for these enzymes in regulating the folic acid-induced inhibition of proliferation, tyrosine kinases play a crucial role in regulating proliferation, differentiation, and transformation of cells (Merlin, Rupasinghe et al. 2021).

Cytotoxic effect of Au NPs on primary liver cancer and colon cancer cell lines

cell proliferation was measured after incubation period for 24 h at 37 °C at concentration of Au NPS (500,250,125,62.5,31.25,15.5) μ /ml the result show there was a significant decrease in the viability of cells ($p < 0.05$) for Au NPs at higher concentration (500,250) μ g/ml for PLCs cell line, while for SW480 cell line all concentrations showed statistically significant decrease in cell viability percent ($p < 0.001$) except 31.5 μ g/ml statistically differs ($p < 0.05$) from control. The cytotoxic effect Au NPs on cells may be due to the higher-level concentration of Au NPS which can interact with other component in the growth media. Cellular media contains vitamins, serum proteins and antibiotics and other chemicals. The high concentration of gold nanoparticles will change their properties such as size, surface chemistry, shape, aggregation state, and surface charge (Alkilany and Murphy 2010). Au NPs at high concentration induce damage to cell membrane, plasma membrane, cellular metabolic activity, mitochondrial activity and a LDH leakage that led to the damage of cells (Ávalos, Haza et al. 2018). Another reason may be due to the oxidative stress as a results of increasing reactive oxygen species (ROS) in mitochondria which is consider toxic to the cells and cause damage to cellular molecules such as DNA, lipids and proteins (Khanna, Ong et al. 2015).

Cytotoxic effect of folic acid and Au NPs on primary liver cancer and colon cancer cell lines

The results of this study showed that all concentrations of both cell lines (PLCs and SW480) decrease cell viability significantly ($p < 0.001$) differ from control the results were closed to these studies (Liu, 2019, Li, 2015) as folic acid led to

improve nanoparticles targeting and exerted its cytotoxic effects against cancer cells proliferation. Gold NPs were directly attached to folic acid can be conjugated to Au nanoparticles by 4-aminothiophenol producing folate-targeted Au nanoparticles (Ramzy, Nasr et al. 2017) in order to avoid significant increases in the overall size and hence properties of the Au NP (Galchenko, Schuster et al. 2019) (Stallivieri, Baros et al. 2015) cytotoxicity of folic acid conjugated gold NPs occur more on colon cancer cell than primary liver cell as colon cancer cells express FARs more than PLCs according to the study (Stallivieri, Baros et al. 2015) so colon cancer cell more sensitive to cytotoxic effect of this conjugation.

Cytotoxic effect of folic acid and Au NPs and laser on primary liver cancer and colon cancer cell lines

The results of this study showed that all concentrations of both cell lines (PLCs and SW480) decrease cell viability significantly ($p < 0.001$) comparing with the control, the results were closed to these studies (Zeinizade, 2018, Beik, 2017). When short laser pulses irradiate Au NPs, a process named nanophotothermolysis occurs. In this process, Au NPs heat up quickly. It has been determined that the absorbed light by Au NP converts to heat on the picosecond time scale. Au NPs are the most promising candidates for nanophotothermolysis since they are strong absorbers, photo stable, nontoxic, and have adjustable optical properties. The absorption maximum of Au NP is tunable from the mid visible region into the infrared region based on the size, shape and material. Au NPs strongly absorb laser irradiation, this absorbed energy transforms quickly into heat, which could cause fatal damage to cancer cells through local overheating effects. Accordingly, Au NPs are potentially very practical and efficient photo thermal agents in therapeutic applications, especially in cancer treatment (Akhter, Ahmad et al. 2012).

The introduction of Au NPs, the most important laser-responsive nanomaterial, into the tissue changes optical properties of the medium and increases local conversion of laser energy into heat. Au NPs possess surface Plasmon resonance (SPR), a well-known property that implies the resonant oscillation of free electrons on the particle surface induced by incident light. Following this phenomenon, the electrons in Au atoms absorb the laser photons and become excited to higher energy levels. Through electron-phonon relaxation, the absorbed photon energy is converted to heat and is transferred into the particle lattice (Manna, Sarkar et al. 2005, Beik, Khademi et al. 2017) To limit such released heat and its resulted thermal damages to the tumor, it is necessary to target Au NPs towards cancer cells using various targeting ligands such as folate, the combination of F-Au NPs with laser exposure leads to selectively heat and destroy the cancer cells (Shakeri-Zadeh, Mansoori et al. 2010).

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