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Photothermal therapy mediated by gum Arabic-conjugated gold nanoparticles suppresses liver preneoplastic lesions in mice

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ABSTRACT

This study validates the utility of Gum Arabic-conjugated gold nanoparticles (GA-AuNPs) and laser to induce photothermal inhibition of hepatocarcinogenesis, via employing a diethylnitrosamine (DEN)-mediated hepatocellular carcinoma model. This work included both of *in vitro* and *in vivo* studies; to investigate the GA-AuNPs cytotoxicity and phototoxicity in hepatic cell line; to delineate the GA-AuNPs therapeutic efficiency in DEN-induced preneoplastic lesions (PNLs) in the liver of Balb-C mice. The therapeutic effects of GA-AuNPs on the mediators of apoptosis, inflammation, and tumor initiation, as well as the histopathological changes in preneoplastic liver have been investigated. Our results infer that GA–AuNPs in combination with laser irradiation led to a significant reduction in the cell viability and in histone deacetylase activity in hepatocarcinoma HepG2 cells. In chemically-induced PNLs mice model our results have demonstrated that GA-AuNPs, with or without laser irradiation, induced cancer cell apoptosis through the activation of death receptors DR5 and caspase-3 and inhibited both of the PNLs incidence and the initiation marker (placental glutathione S-transferase; GST-P). The laser-stimulated GA–AuNPs significantly reduced the tumor necrosis factor-α levels. In summary, GA–AuNPs with laser treatment inhibited liver PNLs via the induction of the extrinsic apoptosis pathway and the inhibition of inflammation.

1. Background

Hepatocellular carcinoma (HCC) is reported affect 500,000 people annually [1]. Hepatocarcinogenesis is the multistep process characterized by several genetic variations causing aberrant growth and malignant transformation of liver cells. Formation of preneoplastic lesions (PNLs) is considered the early step in hepatocarcinogenesis, where part of them developed through time into carcinomas [2]. Thus targeting these lesions can be a successful tool to prevent the development of HCC.

Photothermal therapy (PTT), an efficient minimally invasive approach, involves photothermal sensitizers' usage that can transform photon energy into thermal energy, which can cause irreversible cellular damage, and eventually cell death. For PTT applications, the absorption band for the nanoparticle (NP) sensitizers is preferred in the near-infrared region (NIR > 700 nm) to enhance the light penetration into tissues [3].

Nanotechnology is an uprising area of research that is intensively applied nowadays in the medical field. Gold nanoparticles (AuNPs) have been actively used in PTT [4], where in the presence of NIR radiation, AuNPs produce what is known as plasmon resonance effect (PR) where NPs can absorb and scatter light of wavelengths larger than that of NPs and thus causing a localized heating. This local heating would cause tumor tissue damage and/or release of payload molecules

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of therapeutic importance. The frequency of PR mainly depends on the nanoparticle size and shape [5,7]. The efficiency of AuNPs as PPT is mainly depending on the size, shape and physical properties of these nanoparticles. Nanospheres, nanorods, nanoshells, nanostars, and nanocages have all been reported as subtypes of AuNPs [5]. AuNPs are carriers and enhancers of specific photoactive functional groups to achieve effective penetration across cancer cell membranes. The versatile surface chemistry and unique photophysical characteristics of AuNPs providing a building block for multifunctional nanoplatforms for PTT applications. AuNPs can be conjugated to biologically active moieties for effective localized therapy at tumor sites [7–9]. We have successfully developed a new generation of hybrid AuNPs that demonstrated their cancer cell-targeting features for tumors molecular imaging and therapy using tumor specific nanoprobes [7].

We are currently interested in the utility of Gum Arabic (GA) encapsulated AuNPs (GA-AuNPs) as potential PTT probes, since the highly branched polysaccharide structure of GA consisting of a complex mixture of glycoproteins encompassing arabic acid, with residues of rhamnose, glucuronic acid, galactose, and arabinose, which may promote GA-AuNPs adhesion to the cell surface proteins and maximize its photothermal effects [10]. GA had FDA-approval for human consumption [10]. GA application to achieve *in vitro* and *in vivo* stability for Au and iron oxide NPs were established for imaging and therapy [11], however, PTT of GA-AuNPs has remained relatively unexplored. We herein aim to delineate the photothermal efficiency of GA-AuNPs on the PNLs in the early stage. Our investigations included both *in vitro* and *in vivo* studies; to investigate the GA-AuNPs cytotoxicity and phototoxicity in hepatic cell line; to delineate the GA-AuNPs therapeutic efficiency in diethylnitrosamine (DEN)-induced liver PNLs in Balb-C.

2. Materials and Methods

2.1. Synthesis of Gum Arabic Encapsulated AuNPs (GA-AuNPs)

GA conjugated nanoparticles were synthesized and characterized following our standard protocol [7c–e]. Briefly, 100 μL of 0.1 M $NaAuCl_4$ aqueous solution was added to 6 ml of 12 mg GA aqueous solution, stirred at 80 °C for 10 min. To this mixture, 40 μL of 0.1 M aqueous solution of reducing agent, $P(CH_2NHCOOH)_3$ (THP-Gly; also referred to as 'Katti' peptide) [10] was added. Immediately after, the color changed from yellow into a ruby red, which indicated NPs formation. The metallic core size of GA stabilized AuNPs is 21 ± 6 nm (Fig. 1a–c). By dynamic light scattering instrument (Zetasizer Nano S90, Malvern Instruments Ltd., USA), the GA-AuNPs hydrodynamic size was found to be 78 ± 4 nm thus confirming the GA encapsulation of AuNPs. GA-AuNPs has a negative zeta potential (−35.5 ± 3 mV), an indication of significant *in vitro* stability [7]. Stability measurement that.

involved dilutions in aqueous media, saline, PBS buffer and human serum albumin have confirmed that GA-AuNPs are robust under *in vitro* profiles for potential biomedical applications (Fig. 1d).

2.2. In vitro Application

Human HCC cell line (HepG2; ATCC, Rockville, MD, USA) were routinely cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured in a CO_2 incubator at 37 °C, under humidified environment. Diode laser (Quanta System, Milan/Italy) emitting continuous wave of light was utilized. All *in vitro* exposures were performed in these conditions: wavelength 807 nm [11], average power 500 mW, beam diameter 3.0 cm and power density 50 mW/cm^2. The time period of exposure was 10 min. The laser light was coupled with monocore optical fiber, and the use of a biconvex lens ensured homogeneous exposure of the 96-well plate.

2.3. Cell Viability Assay

To evaluate the phototoxicity as well as the cytotoxicity of GA and GA-AuNP in the presence or absence of laser irradiation, 3,4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide (MTT) cell viability assay was applied. HepG2 cells were cultured in 96-well plate (0.5 × 10 [4] cells/well) for 24 h, and then treated for 24 h with increasing concentrations of GA or GA-AuNP solutions, followed by a 10 min laser exposure. After 24 h, cells were submitted to MTT assay.

2.4. Estimation of Histone Deacetylase Activity

The activity of histone deacetylase (HDAC) was measured in HepG2 cell lysate by colorimetric kit (BioVision, USA) according to the manufacturer's instructions. HepG2 cells were treated with GA (57.7 μg/ml) or GA-AuNP (8.6 μg/ml) with or without laser exposure.

3. *In vivo* Anti-Neoplastic Experiments

Animal experiments were performed adhering to the Ethical Committee guidelines of the National Research Centre, Egypt, and the National Institutes of Health, USA, for the animal care and use. Male wild-type Balb-c mice (18–20 g; 4 weeks old; Theodor Bilharz institute, Cairo, Egypt) were housed in a 12 h light-dark cycle, with free access to sterilized standard chow and water *ad libitum*. Mice were allowed to acclimate for 7 days prior to experiments. Hepatic PNLs were developed in mice using DEN, as previously described by Kushida et al. [12]. A total of 224 mice were randomly subdivided into two large groups: **1.** Normal group (PNLs-free group, control) were subdivided into 6 subgroups (n = 16/group) including control untreated group, laser-, GA-, GA + laser-, GA-AuNPs, GA-AuNPs + laser- treated groups. **2.** PNLs-induced group (PNLs-bearing group, n = 112 mice) received an intraperitoneal (IP) injection of DEN (50 mg/kg b. wt.) every 2 weeks for a total of 12 weeks. At that point, three mice were randomly assigned for a histopathological examination to check hepatic PNLs. After confirming PNLs occurrence, PNLs- bearing mice were classified into 6 subgroups (n = 16/group) including DEN (positive control), DEN + Laser, DEN + GA, DEN + GA + Laser, DEN + GA-AuNPs, and DEN + GA-AuNPs + Laser.

Mice received either an intravenous injection of GA (0.2 mg/100 μl/mouse) or GA-AuNP (30 μg/100 μl/mouse) with or without 10 min laser exposure. The laser source used was the same one used in the *in vitro* study but with an average power of 5 W. The laser energy was delivered to the treatment site in a non-contact mode from the skin surface, where the left region of the mouse abdomen were irradiated by for a single 10 min session. Mice were gross-observed for 4 weeks for any clinical signs. Individual body weights were recorded in day 0 and every week. Weight loss >20% was considered unacceptably toxic. After 4 weeks, mice were anesthetized; blood was withdrawn and the plasma was separated. The livers were excised, rinsed multiple times in ice cold PBS. A liver part was preserved in 4% paraformaldehyde/PBS for histopathological examination, and another liver part (40 mg) was homogenized by grinding in liquid nitrogen and lysed in 1 ml ice-cold lysis buffer (50 mM Tris pH 8, 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulphate, 0.5% Na-deoxycolate monhydrate and protease inhibitor cocktail tablet), and then the lysate was centrifuged at 13,000*g* at 4 °C for 15 min. The supernatant was stored at −80 °C until used for biochemical analyses.

3.1. Histological, Histochemical and Immunohistochemical Analyses

Unbiased histopathological examination (blind to treatment) was performed on the sections of Paraffin-embedded tissues, by hematoxylin and eosin (H & E) staining. To evaluate the cell death mode, liver