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Synthesis and characterization of aminolevulinic acid gold nanoparticles: Photo and sonosensitizer agent for atherosclerosis

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ABSTRACT

Photodynamic and sonodynamic therapies (PDT and SDT, respectively) are emerging as new atherosclerosis treatments. The subsequent generation of free radicals by activated photo and sonosensitizers can lead to apoptotic cell death. The use of gold nanoparticles (AuNPs) as the vehicle for a sensitizer delivery improves reactive oxygen species formation and sensitizer performance. In this study gold nanoparticles functionalized with polyethylene glycol (PEG) were synthesized mixing δ-aminolevulinic acid (ALA) with tetrachloroauric (III) acid in milliQ water solution followed by photo reduction with 300 W xenon lamp. The synthesized ALA:AuNPs were characterized by UV/vis optical absorption, zeta potential and electron microscopy. The mean particle size of spherical ALA:AuNP was ~ 18 nm, with a polydispersity index of 0.437. Singlet oxygen generation efficiency was measured using the trap molecule 1,3-diphenylisobenzofuran. ALA:AuNPs and DPBF were irradiated with 590 nm LED, or pulse ultrasound irradiation (1 W/cm^2 with 1.0 MHz), and consumption of the DPBF was monitored over time by the absorption and emission spectra. The results showed that he gold nanoparticles generate singlet oxygen during light and ultrasound irradiations. THP-1 cells differentiated into macrophages cytotoxicity test were described and was found the half maximal inhibitory concentration (IC50) values ~ 36 nM for ALA:AuNPs. Increase in the fluorescence intensity of PpIX extracted from macrophages incubated with ALA:AuNPs indicating stable encapsulation of ALA into gold nanoparticles and further conversion to PpIX. The potential use of ALA:AuNps as a sensitizer for photo and sonodynamic therapies were investigated. ALA:AuNPs mediated SDT was more effective than PDT. SDT with ALA:AuNPs induced the reduction of macrophage viability in ~ 87,5% in only 2 min. The mechanism underlying SDT-induced apoptosis involves the generation of singlet oxygen. The results indicate that ALA:AuNPs can be used as a novel photo and sonosensitizer for atherosclerosis.

1. Introduction

Photodynamic therapy (PDT) has been used in the regression of atherosclerotic plaques [1,2]. PDT involves the administration of photosensitizer (PS) molecule that accumulates in the macrophages which are involved in development and thrombogenicity of atherosclerosis. Subsequently the excitation of PS with appropriate wavelength and the presence of molecular oxygen results in reactive oxygen species (ROS) that chemically destroy macrophages [3,4]. However, the application of PDT is limited to superficial lesions. Sonodynamic therapy (SDT) derives from PDT and the main difference between SDT and PDT is the energy source used to activate the sensitizers, in this case ultrasound [5]. However, the significant advantage of SDT over PDT is that ultrasound can penetrate deeply in soft tissue [6].

The principal characteristic of a photo or sonosensitizer must have is ability to preferentially accumulate in macrophages and induce a desired biological effect via the generation of cytotoxic species [7]. Protoporphyrin IX (PpIX) is a key molecule in the diagnosis and treatment of diseases such as atherosclerosis and cancer [8]. This is because tissues affected by these diseases have an accumulation of PpIX [9]. It is known that the external administration of δ-aminolevulinic acid (ALA) causes an accumulation of PpIX in the tissues and this fact has been explored in the photodynamic and sonodynamic therapies [10]. Peng et al. demonstrated that the ALA-derived PpIX can be detected to reflect the macrophage content in the plaque [11] and suggested that ALA mediated PDT could reduce macrophage content and inhibit plaque progression, indicating a promising approach to treat inflamed atherosclerotic plaque. Cheng et al. demonstrated that ALA-SDT exhibited synergistic apoptotic effects on THP-1 macrophages, involving excessive intracellular reactive oxygen species generation and

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mitochondrial membrane potential loss [12]. Therefore, ALA-SDT is a potential treatment for atherosclerosis.

The use of gold nanoparticles as carriers of photosensitizers is a very promising approach for PDT and SDT [11]. The association of ALA with gold nanoparticles (AuNPs), increase the applications possibilities of this drug [13–17]. The gold nanoparticles possess a plasmon resonance, a resonant phenomenon where light induces collective oscillations of conductive metal electrons at the NP surface, tunable to different wavelengths by varying the NP size [18]. The AuNPs are very suitable for imaging (MRI, tomography, etc.) and photothermal therapy [19]. The synthesis of nanoparticles are usually carried out by various physical methods like photoreduction [20] and sonification, and chemical methods like co-precipitation [21] and sol-gel technique [22]. Photoreduction synthesis is a clean process which has high spatial resolution, convenience of use, and great versatility [23].

In this study, 5-Aminolevulinic acid (ALA) gold nanoparticles (ALA:AuNPs) functionalized with polyethylene glycol (PEG- were synthetized by photoreduction method and administered to THP-1 macrophage cells to evaluate its toxicity and applications in PDT and SDT.

2. Materials and methods

2.1. ALA:AuNPs solution

The ALA:AuNPs synthesis were performed as previously described [24]. Briefly, 15 mg of $HAuCl_4$ were mixed with 45 mg of ALA and 100 mg of PEG (Poly(ethylene glycol) average mol wt 10,000, Sigma Aldrich) in 100 mL of distilled water at 20 °C. The process was accompanied by vigorous stirring for 5 min, and 10 mL of the resulting solution was exposed to a 300-W xenon lamp for 5 min. The solution pH was adjusted to ~ 7.0.

2.2. ALA:AuNPs characterization

UV–vis absorption spectra were measured by a Shimatzu spectrophotometer, using 1-cm quartz cells.

Shape and sizes of ALA:AuNPs were obtained from a Jeol (Zeiss, Germany) transmission electron microscope. For this, a drop of gold nanoparticles dispersed in distilled water was placed onto a carbon-coated copper grid, the excess liquid was removed using a paper wick, and the deposit was dried in air prior to imaging.

The effective surface charges on the ALA:AuNPs were measured using zeta-potential (Malvern Instruments Zetasizer, Worcestershire, UK).

2.3. Cell culture

Human monocytic leukemia THP-1 cells were cultured in RPMI-1640 medium. Cells were seeded at 5000 cells per well in 96-well plates and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in 95% air. THP-1 cells were treated with 75 nM ng/mL of phorbol myristate acetate (PMA, Sigma-Aldrich Co., St, Louis, MO, USA) for 48 hs to induce differentiation of the cells into macrophages. After differentiation, non-attached cells were removed by aspiration and the adherent macrophages were washed with RPMI-1640 medium 3 times and then incubated in cell culture medium at 37 °C.

2.4. Cell viability assay

PMA pre-stimulated THP-1 cells were incubated with 0–137 nM of ALA:AuNPs for 24 h. Then the cells were washed several times with phosphate buffered saline (PBS) (pH = 7.2–7.6). The ALA-free cells were finally suspended in 500 μL media, and assayed for viability using the colorimetric MTS assay kit based on the CellTiter 96 AQueous One Solution (Promega, Madison, WI, USA), which measures mitochondrial function; the latter correlates with cell viability. In this procedure, the cells were incubated with fresh medium containing MTS reagent for 2 h before measurements at an absorbance of 490 nm. The effect of nanoparticles on cell proliferation was expressed as percentage of inhibition of cell growth relative to the control. Results were statistically compared (ANOVA and Bonferroni post-test) to negative (control cells, NaCl 0.9%) or positive (latex powder suspension, 0.5 g/L - filter sterilized latex extract, 0.5 g/L in culture media, 24 h). The percentage of cell survival was calculated after background absorbance correction and blank absorbance subtraction as follows: % Cell viability = 100 × Experimental well absorbance/untreated control well absorbance.

Table 1
Groups, incubation times and solution concentrations of ALA and ALA: AuNPs. CC (control group).

Groups	Incubation Time (hours)	ALA Concentration nM
(1) ALA:AuNPs	24	68.6
(2) ALA:AuNPs	24	34.3
(3) CC (Cells)	24	–
(4) ALA	4	137.2
(5) ALA:AuNPs	4	68.6
(6) ALA:AuNPs	4	34.3

2.5. PpIX extraction of cells

Around 30.000 cells and 75 ng/mL of PMA were added in each well of a 96 wells plate. After 48 h the macrophages were then divided into groups presented in Table 1.

The contents of each well were collected and added to tubes containing 3 volumes of acetone. These solutions were then centrifuged for 15 min at 4000 rpm, and the supernatants were analyzed in 3 Fluorolog Jobin Yvon fluorimeter. The samples were excited at 400 nm, and emission spectra were measured between 415 and 785 nm. The excitation bandwidth was 5 nm.

2.6. Singlet Oxygen generation

Singlet Oxygen ($^1O_2$) generation was studied from the photodegradation of 1.3-diphenylisobenzofuran (DPBF) [16,17] by absorption and emission spectroscopies. A fresh solution of DPBF (4 μM) in acetone was kept in the dark. For this experiment a 10 mm quartz cuvette containing 1 mL of ALA:AuNPs and 10 μL of DPBF was irradiated with the LED light source at 590 ± 10 nm, InGaN, (VENUS OMEGA-MM OPTICS, Brazil), P~100 mW, by 2 min and removed from the illumination setup to record the absorbance and emission (480 nm with excitation at 422 nm) spectra; this was repeated each 1 min until the sample had been irradiated for 15 min in total.

The singlet oxygen generation induced by irradiation of solutions by ultrasound was studied observing the decrease in the DBPF emission band around 480 nm when solutions were excited at 422 nm. In this case quartz cuvette containing 1.0 mL of ALA:AuNPs and 40 μL of DPBF (4 μM) was irradiated with therapeutic ultrasound HTM Sonic Compact (Brazil). The irradiations were performed with 1 W/cm^2 and 1 MHz frequency. DPBF degradation was evaluated with irradiation from 2 min to 10 min.

2.7. PDT and SDT procedures

The cells followed the protocol described above, and after differentiation into macrophages were incubated with ALA and ALA:AuNPs for 24 h.

For the PDT it was used amber LED at 590 ± 10 nm (VENUS OMEGA-MM OPTICS, Brazil), P~100 mW and exposure time of 2 min.

For the SDT, the transducer of ultrasonic generator, Sonic Compact

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