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Detection of sonoluminescence signals in a gel phantom in the presence of Protoporphyrin IX conjugated to gold nanoparticles

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ABSTRACT

The particles in a liquid decrease the ultrasonic intensity threshold required for cavitation onset. In this study, a new nanoconjugate composed of Protoporphyrin IX and gold nanoparticles (Au–PpIX) was used as a nucleation site for cavitation. The nonradiative relaxation time of Protoporphyrin IX in the presence of gold nanoparticles is longer than the similar time without gold nanoparticles. The acoustic cavitation activity was investigated via recording of the integrated sonoluminescence signal in the wavelength range of 220–700 nm in a gel phantom by a cooled charge coupled device (CCD) at different intensities of 1 MHz ultrasound. In order to confirm these results, a chemical dosimetric method was utilized, too. The recorded sonoluminescence signal in the gel phantom containing Au–PpIX was higher than the other phantoms. These records have been confirmed by the chemical dosimetric data. Therefore, we anticipate that a new nanoconjugate composed of Protoporphyrin IX and gold nanoparticles can act as an efficient sonoluminescence agent and could be introduced as a novel sonosensitizer for sonodynamic therapy.

1. Introduction

When a liquid is irradiated with high intensities (>1 W) and low frequencies (⩽1 MHz) of ultrasound, acoustic cavitation occurs [1]. Acoustic cavitation can occur as stable and transient modes. In a stable mode, the bubbles oscillate around an equilibrium radius during a considerable number of acoustic cycles without collapsing. In transient cavitation, bubbles grow rapidly and expand up to several times their original size, and violently collapse during a single acoustic compression cycle [2]. In fact, during the collapse, very high shear stresses and shock waves are produced. Moreover, very high pressure and temperature at the collapse region can produce free radicals, erosion, emulsification, molecular degradation and sonoluminescence. This type of cavitation can be fatal to cells and is utilized to destroy cancer tumors [3].

The combination of ultrasound with a sonosensitizing agent has been studied as a means of increasing the efficacy of cancer treatment (sonodynamic therapy). In sonodynamic therapy, after selective accumulation of the sonosensitizing agent in tumors, cytotoxicity is induced during the activation of the mentioned agent by ultrasonic exposure [4].

Protoporphyrin IX (PpIX) is an efficient hydrophobic sensitizer which is activated by both light and ultrasound waves [5]. The subsequent interaction of activated PpIX with molecular oxygen produces cytotoxic reactive oxygen species (ROSs), particularly singlet oxygen ($^1O_2$), which causes irreversible destruction on the target tissue [6].

The activation of a sensitizer is dependent on cavitation, and therefore, high-intensity ultrasound is an important necessity. The use of high-intensity ultrasound is one of the existing challenges in sonodynamic therapy. Furthermore, high intensity ultrasound can also elicit bioeffects on healthy tissues in the peritumoral region [7].

On the basis of a few reports, the existence of a particle in a liquid provides a nucleation site for the cavitation bubble because of its surface roughness and leads to decrease in the cavitation threshold responsible for the increase in the quantity of bubbles, when the liquid is irradiated by ultrasound [8,9]. Thus, in this context, one approach is based on providing the nucleation sites which participate in the formation of cavities to reduce the threshold intensity required for cavitation.

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There are many problems regarding the clinical application of existing sensitizers. For example most sensitizer molecules are hydrophobic and can be easily aggregated in aqueous media [10]. The sensitizer aggregation will decrease its efficiency for singlet oxygen production. Also, the aggregated sensitizer cannot be simply injected intravenously [10]. To design an efficient sensitizer, there has been increasing interest in using nanoparticles as sensitizer carriers. Nanomaterials are promising for the following reasons: (1) they can be made hydrophobic; (2) they have an enormous surface area, and their surface can be modified with functional groups possessing a diverse array of chemical or biochemical properties [10]; and (3) owing to their subcellular and submicrometer size, nanoparticles can penetrate deeply into tissues and are generally taken up efficiently by cells [8].

Gold nanoparticles (GNPs) have been characterized as novel nanomaterials for use in cancer therapy because of their special optical properties [11,12]. Their low toxicity, good uptake by mammalian cells, and antiangiogenetic properties make GNPs highly attractive for medical applications [13].

The nonradiative relaxation time of PpIX in the presence of GNPs is longer than the similar time for PpIX without GNPs [14]. This long relaxation time is very favorable for the efficient generation of singlet oxygen.

In this study, the cavitation potential of PpIX conjugated to GNPs (Au–PpIX) has been studied via two methods of sonoluminescence detection [15] and chemical dosimetry [16] at therapeutic intensities of ultrasound. Sonoluminescence detection within tissues would not be possible, in consideration to their opacity deficient. As Daniels and Price had purposed a phantom from agar gel for modelling of biological systems due to its physical and chemical properties on soft tissues against ultrasound [17], the present study was performed on agar gel phantom. Also, in most studies, the sonoluminescence signal has been detected by a photomultiplier tube (PMT) [17]. The technical problems related to PMTs, such as difficulties related to its appliance, expensive cost of required tools and the need for supplementary tools like storage oscilloscope and specialized software, let us to the use of a cooled charge coupled device (cooled CCD) as a sonoluminescence monitoring device [18,19].

When water is sonicated, OH radicals are formed on thermolysis of H_2O. Simplified equations for production of free radicals by collapse of cavitation in water solutions are shown in the following equations [20]:

$$H_2O \rightarrow H^{\cdot} + {}^{\cdot}OH \quad (1)$$

$$H_2O \rightarrow H_2 + {}^{\cdot}O \quad (2)$$

$$H^{\cdot} + H_2O \rightarrow H_2 + {}^{\cdot}OH \quad (3)$$

$$O + H_2O \rightarrow {}^{\cdot}OH + {}^{\cdot}OH \quad (4)$$

Such chemical products may also be used in measuring cavitation activity. It has been shown that terephthalic acid (TA) [benzene-1, 4-dicarboxylic acid] is suitable for detecting and quantifying free hydroxyl radicals generated by the collapse of cavitation bubbles in ultrasound irradiations. During this process, TA solution as a dosimeteric solution reacts with a hydroxyl radical generated through water sonolysis. Therefore, 2-hydroxyterephthalic acid (HTA) is produced which can be detected using fluorescence spectroscopy with excitation and emission wavelengths of 310 and 420 nm, respectively [21].

2. Materials and methods

2.1. Preparation and characterization of Au–PpIX

Protoporphyrin IX (Sigma–Aldrich, Munich, Germany) was conjugated [22] to the GNPs through a bidentate linker according to a recently reported method [22]. Characterization of the GNPs was determined by two techniques: UV–visible spectrophotometry and transmission electron microscopy. The nanoparticles' shape was nearly spherical with an average diameter of 7 nm [22].

2.2. Recording of sonoluminescence signals

2.2.1. Ultrasound generator system

Ultrasound irradiation was conducted with a therapeutic ultrasound unit (215A; coproduct of Novin Medical Engineering Co., Tehran, Iran; and EMS Co., Reading, Berkshire, England) in a continuous mode at a frequency of 1.1 MHz with a maximum intensity of 2 W/cm^2 for 3 min. Acoustic calibration for the power of the device was performed in a degassed water tank, using an ultrasound balance power meter (UPM 2000; Netech Corporation, Grand Rapids, MI) with uncertainty of ±1 mW. All quoted intensities were spatial average-temporal average in our experiments. An ultrasound transducer with a surface area of 7.0 cm^2 was horizontally submerged in the bottom of a glass container filled with degassed water.

2.2.2. Agar gel preparation

The gels were prepared by adding Agar (Sigma) to half the final volume of stirred water. The mixture was covered, continually stirred and gradually heated up to 90 °C in water bath, where a clear solution was formed. The hot solution was allowed to cool down to near 50 °C and made up to the final volume with adequate distilled water (buffered to pH 9), which had been warmed up to near 50 °C. The solution was poured into molds ($4 \times 4 \times 6$ cm^3) and any visible bubbles were removed. The gels were allowed to reach dissolved gas equilibrium by overnight standing at normal room temperature (typically 15–17 h). Gel concentration was selected as 0.75% (w/v) [17].

2.2.3. Design and construction of a phantom

A container with dimensions of $10 \times 8 \times 16$ cm^3 was constructed from black Perspex (PMMA) slabs of 5 mm thickness. A quartz window was designed in one side of the container. A 400 μm fiber optic equipped with special connectors was used for light transfer from the phantom to the spectrometer. The location of the ultrasound probe was considered under the phantom through a specially designed hole in the floor of the container. The container was filled with degassed distilled water thermostated at 18 °C. In order to prevent successive reflections of ultrasound waves, a layer of foam was pasted inside the container on the floor and the walls. Black Perspex slabs sealed the phantom from background light. Cavitation detection was conducted in a phantom made from a transparent agar gel. In order to produce comparable values for the light emissions from the gel, percentage of transmission for the wavelength in the range of 200–900 nm for the gel was measured for a standard 1 cm path length using a UV spectrophotometer [17]. Transmission spectrum of agar gel is shown in Fig. 1.

In this study, the experiments were performed on four similar blocks of gel: gel containing PpIX (0.12 mg/ml), gel containing GNPs (0.22 mg/ml), gel containing Au–PpIX (0.39 mg/ml) and simple gel. It should be noted that PpIX, GNPs and Au–PpIX were injected into the gel's blocks, 5 min before measurement.

2.2.4. Cooled electro-optic CCD

The sonoluminescence signal was detected using a cooled electro-optic spectrometer (Thermo-Electric cooled and regulated CCD, Avantec Co., NL-6961 RB Eerbeek, Netherlands). The AvaSpect-2048 × 14 Fiber Optic Spectrometer is a back-thinned type CCD spectrometer with high quantum efficiency and high UV sensitivity. The spectrometer was equipped with a fiber optic entrance connector (standard SMA) collimating and focusing mirror and a diffraction grating.