Journal of Photochemistry and Photobiology B: Biology 130 (2014) 40–46

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Gold nanorods-bombesin conjugate as a potential targeted imaging agent for detection of breast cancer

Zahra Heidari [a], Reyhaneh Sariri [a,*], Mojtaba Salouti [b,*]

[a] Department of Biology, University of Guilan, Rasht, Iran
[b] Biology Research Center, Zanjan Branch, Islamic Azad University, Zanjan, Iran

ARTICLE INFO

Article history:
Received 20 July 2013
Received in revised form 26 October 2013
Accepted 31 October 2013
Available online 15 November 2013

Keywords:
Breast cancer
Photoacoustic imaging agent
Targeting
GRP receptor
Bombesin

ABSTRACT

Photoacoustic imaging (PAI) is a hybrid biomedical imaging modality that offers both strong optical absorption contrast and high ultrasonic resolution. PAI is capable of *in vivo* molecular imaging, thus facilitating further molecular and cellular characterization of cancer. In this study, Gold nanorods (GNRs) were synthesized and coated with polyethyleneglycol (PEG). Then, the PEG-GNRs were conjugated with bombesin (BBN), a cancer seeking peptide, for production of a potential photoacoustic targeting imaging agent for detection of breast cancer. The optical property, biocompatibility, stability and *in vitro*/*in vivo* binding affinities of GNR-PEG-BBN for breast cancer cells were investigated. UV–vis spectroscopy confirmed the conjugation of bombesin with PEG-coated GNRs. The stability assessment proved high optical stability of GNR-PEG-BBN in human blood serum up to 12 h. Cytotoxicity study showed biocompatibility of GNR–PEG–BBN conjugate. Molecular targeting ability was approved in cells over expressing gastrin-releasing peptide (GRP) receptor (breast cancer cell line) in comparison with cells that do not express GRP receptor (skin fibroblast cell line). The selective accumulation of GNR-PEG-BBN was demonstrated in breast tumor in comparison with unconjugated gold nanorods, following the intravenous administration of GNR-PEG-BBN to breast tumor-bearing mice. This study demonstrated the potential of GNR-PEG-BBN as a photoacoustic imaging agent that can provide improved specificity and sensitivity for breast cancer detection.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Breast cancer has become one of the leading causes of death in women today [1]. Many researches are ongoing to develop more effective imaging methods for early diagnosis of breast cancer [2]. Molecular imaging refers to remote sensing the characteristics of biological process and interactions at the molecular level [2–4]. It has a great potential for early detection, because aberrations at the cellular and molecular levels occur much earlier than anatomic changes [4]. However, high resolution molecular imaging has been limited to relatively shallow penetration depth that can be accessed with microscopy [5]. The need for an imaging technique that can provide high optical contrast image in microscale resolution and at a reasonable penetration depth has now been filled with photoacoustic imaging (PAI) [5–7]. In this technique, non-ionizing laser pulses are delivered into the biological tissues. Some delivered energy is absorbed and converted into heat, leading to transient thermoelastic expansion and thus wideband ultrasonic emission [5–7]. The generated ultrasonic waves are then detected by ultrasonic transducers to form the image [5–7].

The recent studies are focused on utilizing gold nanorods (GNRs) as a contrast agent to enhance the image contrast in PAI [7–9]. GNRs can absorb light about one thousand times more strongly than an equivalent volume of an organic dye and their plasmon resonance absorption and scatter in the near infrared region makes them suitable for *in vivo* imaging applications [7–9]. Jokerst and co-workers reported (2012) that gold nanorods, under *in vivo* conditions, exhibit significant photoacoustic contrast and increase the diagnostic power of photoacoustic imaging modality [9]. Advances in the field of molecular biology (i.e., Discovery of various disease specific biomarkers) have prompted the development of site-specific contrast agents (active targeting) that can provide molecular information in the context of a high resolution anatomical map of the body [4,10]. Active targeting takes advantage of the fact that cancer cells over-express certain receptors on the cell surface [4]. Many active targeting strategies use the enhanced permeability and retention (EPR) effect, so that active and passive targeting mechanisms act synergistically that lead to

* Corresponding authors. Address: Namjoo St., Department of Biology, University of Guilan, Rasht, Iran. Tel./fax: +98 131 3233647, mobile: +98 9111325824 (R. Sariri). Address: Moallem Street, Etemadieh, Zanjan Branch, Islamic Azad University, Iran. Tel./fax: +98 241 4224024, mobile: +98 9121454954 (M. Salouti).
E-mail addresses: sariri@guilan.ac.ir (R. Sariri), saloutim@yahoo.com (M. Salouti).

1011-1344/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jphotobiol.2013.10.019

higher concentration of nanostructures in the cancer region than that in healthy tissues [4,10].

Motamedi and co-workers showed that Herceptin-gold nanorods conjugate, under *in vivo* conditions, exhibit significant optoacoustic contrast and increase the diagnostic power of optoacoustic imaging modality [11]. The nature of peptide itself makes it an attractive compound to act as a bullet targeted at the corresponding peptide receptors. Peptides used for tumor targeting, show some advantages over antibodies: peptides are small, high affinity and show rapid diffusion into (target) tissues resulting in rapid pharmacokinetics. The fast blood clearance could lead to a high tumor to background ratio shortly after administration [12,13]. In addition, the peptides can tolerate harsh chemical conditions and are easy to purify and modify peptide receptors [12,13].

Bombesin (BBN) and its human counterpart gastrin-releasing peptide (GRP) belong to a family of brain-gut peptides, has shown, in addition to the physiological effects, to play an important role in cancer growth [13]. Peptides GRP receptors are over expressed in many cancers including breast, prostate and small cell lung cancer [12–17]. The low expression of BBN/GRP receptors in normal tissues and relatively high expression in a variety of human tumors can, therefore, be of biological importance and form a molecular basis for BBN/GRP receptor mediated PAI imaging [12–17]. In this study, we used a BBN analog derived from the universal binding sequence (KGGCDFQWAV-βAla-HF-NIe), which was known to bind to all four receptor subtypes [13]. This analog was previously used only in a nuclear medicine study and there is not any report about its using in a nano-medicine study [13]. So, the objective of present study was the conjugation of bombesin with PEG-coated GNRs as a specific GRP receptor contrast agent for subsequent application in photoacoustic imaging.

2. Experimental

2.1. Material

Chloroauric acid ($HAuCl_4$.3H2O), cetyltrimethylammonium bromide (CTAB), ascorbic acid, fetal bovine serum (FBS), RPMI 1640, trypan blue, streptomycin, penicillin and trypsin–EDTA, analytical grade nitric and hydrocholoric acid were purchased from Sigma (St. Louis, MO, USA). Sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$) and dithiothreitol (DTT) were purchased from Merck, Germany. Thiol-PEG-carboxylate-5000 (COOH-PEG-SH) was purchased from Nanocs, USA. The human breast cancer cell line (T47D) and the human skin cancer cell line (E8 (AG01522) were purchased from Pasteur Institute, Tehran, Iran. Bombesin (BBN) peptide synthesis was ordered to GenScript Company (USA).

2.2. Synthesis of gold nanorods

GNRs with a peak absorption wavelength of 780 nm were prepared via the seeded-growth mechanism previously described by Eghtedari and co workers [5,11]. Briefly, gold seed was prepared by the addition of 7.5 ml of 0.1 M CTAB to 0.25 ml of 0.01 M gold chloride. Then, 0.6 ml of 0.01 M $NaBH_4$ (prechilled for 10 min in an ice water bath) was quickly added and the mixture was shaken for 2 min. The growth mixture was prepared with the following: 4.7 ml of 0.1 M CTAB, 0.2 ml of 0.01 M gold chloride and 45 μl 0.01 M $AgNO_3$. The solution was yellow/brown, but became translucent upon the addition of 32 μl 0.1 M ascorbic acid. Then, the seed solution (21 μl) was added. The solution color was changed to purple/brownish over 60 min. The size and morphology of GNRs were analyzed by transmission electron microscopy (TEM) using a Hitachi HF-2000 field emission high-resolution TEM operating at 200 kV. The particles were then characterized for zeta-potential and UV–vis light absorption. Measurements were made in triplicate for each sample.

2.3. Biofunctionalization of gold nanorods

Five ml of CTAB-stabilized GNRs was centrifuged twice in a 1.5 ml eppendorf tube at 10,000 rpm for 15 min and resuspended in 5 ml of deionized water [11]. An amount of DTT was added in the solution with molar ratio DTT/HS-PEG-COOH about 1/1 [18,19]. The solution DTT/HS-PEG-COOH then was added into 5 ml of GNRs and the mixture was incubated at room temperature for 24 h and dialyzed using cellulose ester membrane dialysis (Spectrapor) for 12 h against PBS buffer [11–19]. The final solution was again centrifuged at 5500 rpm for 15 min to remove any excess HOOC-PEG-SH [11–19]. Next, bombesin immobilized covalently onto the PEG coated GNRs surfaces [11]. EDC and sulfo-NHS was added to 5 ml of PEG coated GNRs at a final concentration of 4 mM and 1 mM, respectively [11]. The mixture was sonicated for 25 min at 4 °C to produce activated GNRs (GNRs that are capable of binding to the amine side chain of bombesin). Bombesin was added to a final concentration of 500 mg/ml to 5 ml of activated GNRs. The mixture was sonicated at room temperature for 2 h [11]. After successful conjugation, the unbound peptide was separated by centrifugation at 12,000 rpm for 10 min [14]. The final solution was diluted by adding PBS to achieve an optical density of 1.0 at 780 nm. A schematic is given in Fig. 1 to simply illustrate the synthesis process of the functionalized GNRs.

2.4. GNR–PEG–BBN characterization

The physical chemistry property of the conjugates was monitored through optical and zeta potential measurements to confirm surface chemistry [11,13,18–20]. UV–vis spectrophotometer (CARY-100 BIO, Varian, USA) was used for investigating the interaction of bombesin with gold nanorods. The spectrum ranged from 200 to 900 nm at the resolution of 1 nm [11,13,18–20]. As a means to confirm the altered nanorod surface chemistry at various stages, zeta potential measurements (Malvern Zetasizer Nano, UK) were performed on gold nanorods in the original CTAB, after stabilization with HOOC-PEG-SH, and finally after bombesin conjugation [14,18].

2.5. Optical stability of the bioconjugate in human serum

To verify the stability of GNR-PEG-BBN in human blood serum, the conjugate was added to 1/1 ratio of human blood serum/PBS (total volume 2 ml, GNR concentration 0.02 μM) [21]. The optical absorbance of solution at 800 nm was monitored at multiple time points for 12 h. The control solution was included human blood serum and PBS only (1/1 ratio) [21]. Subtracting the "serum" absorbance from "the conjugate in serum" absorbance shows GNR-PEG-BBN absorbance. If the conjugate absorbance remained intact around 800 nm, it is indicated that the conjugate is stable in human blood serum [21].

2.6. Cytotoxicity study

Cell viability of T47D cell line (human breast cancer cell line) with GNR-PEG-BBN, GNR-PEG and GNR-CTAB was determined using trypan blue test [11]. T47D cells were harvested and resuspended at 1×10^4 cells/200 μl in RPMI-1640 medium and cultured in 24-well plate. After 24 h, 100 μl (~100 μg/ml) of each sample (GNR-PEG-BBN, GNR-PEG and GNR-CTAB) was added to the wells [11]. 100 μl PBS buffer was added to 6 wells of plate as control too. After 24 h incubation, the living/dead cells were visualized

Download English Version:

https://daneshyari.com/en/article/30419

Download Persian Version:

https://daneshyari.com/article/30419

Daneshyari.com