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Rapid synthesis of ^{125}I integrated gold nanoparticles for use in combined neoplasm imaging and targeted radionuclide therapy

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HIGHLIGHTS

- Effects of gold nanoparticles in mitochondria and fragmentation.
- Mechanism of increased EPR by AuNPs and PEG-AuNPs.
- Production and characterization of ^{125}I doped AuNPs as a theranostic agent.
- Establishment of probability density function for dosimetry estimations.

ABSTRACT

The selective delivery of radionuclides to tissues of interest remains a problematic task during treatment. The lack of tissue specificity for many therapeutics limit their efficacy by putting healthy organs and tissues at risk (e.g., side effects). Therefore, high specificity therapeutic strategies are needed to overcome these risks. The objective of this study was to use a modified citrate reduction technique to synthesize gold nanoparticles (AuNPs) containing ^{125}I in order to combine their unique therapeutic and diagnostic properties. This task was accomplished by varying the insertion time of ^{125}I, which will cause complete aggregation if added too early in the AuNP synthesis process. Even though ^{125}I was utilized in this experiment, studies are underway to see if this approach can be extrapolated to shorter-lived isotopes (e.g., ^{211}At). Characterization of the ^{125}I-AuNPs was carried out using UV–Vis spectrometry and Transmission Electron Microscopy (TEM). The appropriate addition time of ^{125}I was determined to be approximately 50 s after the addition of sodium citrate. TEM measured the nanoparticles' diameters to be in the 10–20 nm range. The AuNPs were found to be extremely stable, with no observable leaching of radioactivity into the solution. ^{125}I-AuNPs could be beneficial as a contrast agent in CT imaging and therapy since AuNPs enhance the bio-delivery of ^{125}I to neoplasms.

1. Introduction

1.1. Current obstacles

The selective bio-delivery of cytotoxic agents to target cancer cells with minimal adverse effects on healthy tissues remains a challenge when developing therapeutics (Emami et al., 1991; Stone et al., 2003). Gold nanoparticles (AuNPs) have gained recognition as enhancers of therapeutic bio-delivery and diagnostic imaging, since they have the remarkable ability of matching the pathophysiology of tumors, increasing therapeutic delivery while also sparring healthy tissue (Lim et al., 2011; Shukla et al., 2012). This makes the conjugation of radiolabeled compounds to AuNPs an efficacious route of treatment to study. As will be discussed in this introduction, functionalized AuNPs and single radiolabeled compounds have been extensively investigated individually in diagnostic imaging and therapy. Each approach when utilized separately still carry confounding issues associated with their imaging potential, biological half-lives, retention in target tissues, and degree of infiltration into tissues of interest. However, their combined utility as a theranostic has not been assessed extensively. Therefore, by combining radionuclides and AuNPs a number of limitations could potentially be overcome.

1.2. Enhanced permeability and retention

First, AuNPs offer unique opportunities in the delivery of

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biologically relevant compounds (e.g., radionuclides) due to the enhanced permeability and retention (EPR) effect in neoplasms (Ghosh et al., 2008; Peer et al., 2007). As of yet, the mechanism of EPR, beyond the enhanced permeability of the tumor vasculature aiding the effect, hasn't been fully or extensively explained in current literature. Once the AuNPs are within the neoplasm, it is believed they can enter cells via macropinocytosis or some other form of specialized endocytosis, with or without involvement of surface receptors. We suggest that the enhanced retention of AuNPs in cancer cells may also be caused, in part, by the interaction of AuNPs with the mitochondria as a result of mitochondrial dysfunction. Mitochondrial dysfunction has already been linked to cancer progression (Warburg, 1956). The main evidence was the switch from aerobic to anaerobic respiration, which results in a significant increase in reactive oxygen species (ROS) in neoplastic cells (Wallace, 2005a). Mitochondrial dysfunction has also been characterized by increases in mitochondrial fission, resulting in excess of either long chain or clustered mitochondrial fragments frequently seen in neoplastic cells (Alirol and Martinou, 2006; Wallace, 2005b; Zhao et al., 2013). More recently, studies have also found that the vitiation of ATP synthesis due to mitochondrial uncoupling in response to perversions of the mitochondria's membrane potential, can promote the Warburg effect, potentially contributing to the chemoresistance of leukemia cells (Samudio et al., 2009). Therefore, to elaborate further we suggest that mitochondrial dysfunction leads to differences in mitochondrial membrane and/or surface potentials (i.e., electrostatic potentials), which potentially enhance the EPR effect via attraction.

To give credence to membrane and surface potentials enhancing the EPR effect, various studies have shown considerable internalization of AuNPs by cancer cells followed by their incorporation with the mitochondria; in some instances this mechanism was even used to try and induce apoptosis or radio-sensitization of cancer cells (Mkandawire et al., 2015; Taggart et al., 2014; Wang et al., 2011a; Yang et al., 2015). Many other studies validate this assessment by showing the considerable impact of mitochondrial surface potential on the uptake and even the potential cytotoxicity of gold nanoparticles (Fröhlich, 2012; Zhao et al., 2012). Varying the surface charges of the nanoparticles could therefore be the key to targeting the cancer stem cell sub-populations. For example, Liu et al. demonstrated size and surface charge effects on gold nanoparticles interactions with phagocytic and nonphagocytic cells, RAW 264.7 and HepG2 cells, respectively (Liu et al., 2013). Due to the phagocytic nature of cancer stem cells, the use of negatively charged nanoparticles may drive up the cancer stem cell's uptake of nanoparticles preferentially over the non-phagocytic cells of healthy tissue. This is also seen with the metastatic and highly-invasive B16F10 melanoma populations, which take up nanoparticles readily and distributes them into their golgi apparatuses and endoplasmic reticulums (Chang et al., 2008).

The importance of mitochondrial fragmentation in cancer progression is emphasized by studies showing that induced fusion of fragmented mitochondria resulted in cell cycle arrest in lung cancer (Rehman et al., 2012). The quantity of mitochondrial fragmentation can be directly linked to the exacerbation of lamellipodia formation resulting in increased motility/protrusion, driving the metastatic potential of cancer cells (Rehman et al., 2012). On the other hand, polyethylene glycol (PEG), which is also one of the main coating agents used during gold nanoparticle synthesis, has been shown to induce mitochondrial fusion by reducing the mitochondria's membrane potential (Robinson et al., 1979; Wojcieszyn et al., 1983). PEG-AuNPs reduce the surface potential of the fragmented mitochondria, causing fusion around the AuNPs; therefore, PEG properties may also be some of the key reasons why PEG-AuNPs have enhanced retention in neoplastic cells. This enhanced retention is corroborated by a study where PEG-AuNPs were able to reduce the surface potential of fragmented mitochondria, induce fusion, arrest cell cycle, and then induce apoptosis in chronic myeloid leukemia cells (Huang et al., 2014). Reducing the surface potentials of the fragmented mitochondria would potentially result in either a situation where the mitochondria fixes its metabolic activity or depletes its capability of supplying ATP at the quantities required by the neoplasm, which would explain PEG's dramatic ability to induce apoptosis in colon cancer cell lines (Roy et al., 2001).

1.3. The radiochemical properties of ^{125}I

The radiological properties of ^{125}I have facilitated its extensive use in brachytherapy for the treatment of skin, prostate, lung, and brain tumors, while also being used in certain situations for thyroid imaging (anatomic and physiologic function) and as a label in radioimmunoassays (Al Mahmoud et al., 2008; Beydoun et al., 2014; Chen et al., 1999; Corrie et al., 1981; Garretson et al., 1987; Goddard et al., 1986; Gutin et al., 1984; Harper et al., 1961; Prince et al., 1979). It has also been used with moderate success in the treatment of multiple recurrent solid tumors (Gaspar et al., 1999; Ling, 1992).

The half-life of ^{125}I is 60.14 days and decays by electron capture (EC) to the excited state of ^{125}Te, which immediately emits a 35.5 keV gamma ray (Friedlander and Orr, 1951). This is followed by the emission of short-range, highly localized, low-energy conversion and Auger electrons. Conversion electrons are ejected with a maximum energy of 35 keV while the 21 typical Auger electrons produced per decay possess energies ranging from 0.07 to 30.1 keV (Silberstein, 2012). Auger-electron emissions have also been found very effective for therapy and diagnostics (Stepanek et al., 1996; Wieland et al., 1981).

The Auger effect is the primary process by which ^{125}I decays and produces cellular damage (Hofer, 1996; Hofer et al., 1992; Kassis, 2004). When ^{125}I decays it produces an energy deposition of approximately 106 cGy, in a 2 nm radius spherical volume surrounding the point of decay (Balagurumoorthy et al., 2012; Humm et al., 1995). Based on the energies of the electrons produced by ^{125}I, the electrons will approximately travel 100 nm in water and only 10 nm in gold (Piotr et al., 2013; Vaughan, 1986). This range will limit the effective diameters of the AuNPs to 20 nm so that radiative emissions from ^{125}I, potentially at the core of the AuNPs, will be able to reach outside the AuNP (Dulkeith et al., 2004; Hangleiter and Hacker, 1990; Zhang and Yates Jr, 2010).

While Auger electrons possess relatively low-energies, their cytotoxic potential parallels that of high LET radiation (e.g. alpha particles) when their emission occurs in close proximity to DNA molecules (Bradley et al., 1975; Hofer, 2000; Hofer and Hughes, 1971). As a result, the electron decay of ^{125}I produces DNA double strand breaks and cluster damage, detectable up to 70 angstroms from the decay site, making it a very potent therapeutic if in close proximity to the nucleus or organelles (Balagurumoorthy et al., 2012; Martin and Haseltine, 1981). Moreover, the Auger electron cascade in gold serves as a key benefit to the synthesis of ^{125}I-AuNPs, as the effect potentially enhances the radionuclide's cytotoxicity (Rahman et al., 2009). Auger cascades and the highly localized deposition of energy around the AuNPs are key features of how AuNPs influence the mitochondria and radiosensitization (Taggart et al., 2014).

1.4. Combining AuNPs with radionuclides

A major impediment to the use of ^{125}I in its free state, like all other isotopes of iodine, is that it has very little affinity for tissue other than the thyroid, even in the presence of a tumor (Lundh et al., 2006). Therefore, seeds and various other carriers are utilized to mitigate this issue and allow for targeting specific areas of interest. Utilizing AuNPs would potentially increase the efficacy ^{125}I not only as a therapeutic agent in tumors of the thyroid, but for other primary and secondary neoplasms all over the body. This is a property that other therapies such as brachytherapy do not have the ability to perform; it may target specific tumors after surgical intervention but since it does not circulate in the blood there is no means of targeting CTCs or sites of metastasis besides potential immune responses (i.e., bystander effects).