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Evaluation of ^{99m}Tc-glucarate as a breast cancer imaging agent in a xenograft animal model

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Abstract

Introduction: The use of [^{99m}Tc]glucarate has been reported as an infarct-avid agent with the potential for very early detection of myocardial infarction. [^{99m}Tc]Glucarate has also been postulated as an agent for non-invasive detection of tumors. The aim of our study was to develop a Glucarate kit and evaluate [^{99m}Tc]glucarate as a potential cancer imaging agent in female SCID mice bearing human MDA-MB-435 breast tumors.
Methods: Glucarate in a kit formulation was labeled with ^{99m}Tc and evaluated for radiolabelling efficiency and radiochemical purity. The Glucarate kit stability was assessed by monthly quality controls. The pharmacokinetics of [^{99m}Tc]glucarate were determined in female SCID mice bearing MDA-MB-435 human breast carcinoma tumors at 0.5, 1, 2, 4 and 24 h. Nuclear imaging studies were performed with a micro-single photon emission tomography (SPECT)/computed tomography (CT) system at 2 h post injection, while magnetic resonance imaging (MRI) was employed for tumor morphology analysis and metastatic deposit localization.
Results: The Glucarate kits exhibited a stable shelf life of 6 months. [^{99m}Tc]Glucarate was obtained with radiochemical purity greater than 95%. Biodistribution studies demonstrated moderate tumor uptake coupled with high renal clearance. Tumor-to-muscle ratios were 4.85 and 5.14 at 1 and 4 h post injection. MRI analysis showed tumors with dense cellular growth and moderate central necrosis. [^{99m}Tc]Glucarate uptake in the primary MDA-MB-435 shoulder tumors and metastatic lesions were clearly visualized with micro-SPECT/CT imaging.
Conclusions: Selective tumor uptake and rapid clearance from nontarget organs makes [^{99m}Tc]glucarate a potential agent for breast cancer imaging that awaits validation in a clinical trial.

Keywords: Breast cancer; Diagnosis; ^{99m}Tc; Glucarate; Radiopharmaceutical; SPECT imaging

1. Introduction

Among women, breast cancer is the most common cause of cancer mortality, accounting for 16% of cancer deaths in adult women [1]. Nuclear medicine procedures for breast cancer diagnosis have been primarily based on the use of ^{99m}Tc-sestamibi imaging [2]. However, ^{99m}Tc-sestamibi imaging was compromised in tumors expressing the multidrug resistance (MDR) phenotype and in small lesions [3]. Tumor cells that overexpress the MDR gene were able to pump ^{99m}Tc-sestamibi out of the cell, reducing the imaging of MDR positive tumors [4,5]. Moreover, the imaging sensitivity of ^{99m}Tc-sestamibi in tumors less than 1 cm and in lesions with low desmoplastic activity was reduced [6–8]. Radiopharmaceuticals that could detect tumors and would not be affected by multi-drug resistance (MDR) phenotype are of great interest. Although positron emission tomography (PET) imaging has set a new standard in oncology, 2-deoxy-2-(^{18}F)fluoro-D-glucose (FDG) has not proven to be

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effective in the detection of small, low-grade, breast tumors [9]. In many cases, ^{99m}Tc-based imaging offers a lower cost and greater availability alternative to PET.

Glucarate, a normal product of the metabolism of D-glucuronic acid, is a low-molecular-weight six-carbon dicarboxilic acid that is eliminated in the urine in quantities of 10 mg/day and has been used as a stabilizer of calcium gluconate injections for years. Glucarate can be radiolabelled with sodium pertechnetate Na[$^{99m}TcO_4$], resulting in [^{99m}Tc]glucarate [10]. It has been reported that Glucarate binds to the histones of necrotic myocardium [11–13] as well as exhibiting tumor accumulation [14–16]. [^{99m}Tc]Glucarate was shown to accumulate in SCID mice xenografted with human breast tumors [14]. Importantly, [^{99m}Tc]glucarate uptake was shown to be similar in MDR positive and negative MCF7 tumor xenografts, indicating that its localization is not affected by the MDR phenotype [16]. Tumor uptake of [^{99m}Tc]glucarate was shown to be enhanced under hypoxic conditions [17,18]. More recently, [^{99m}Tc]glucarate uptake was reported to be higher in necrotic cells than apoptotic cells [17]. In apoptotic cells, [^{99m}Tc]glucarate was distributed throughout the cytosol and nuclear fractions, while in necrotic cells [^{99m}Tc]glucarate was found primarily in the nucleus. Although the exact mechanism(s) by which glucarate localizes in tumors remains unknown, a fructose transporter appears to be involved in viable cells [19]. It has also been postulated that the loss of membrane integrity associated with necrosis facilitates intracellular [^{99m}Tc]glucarate diffusion, where it is attracted to the nucleus by histone binding [12,13,16,17]. It appears that different mechanisms of [^{99m}Tc]glucarate tumor uptake may be correlated with cell integrity and the tumor microenvironment, distinguishing the degree of tumor necrosis. Imaging the degree of tumor necrosis by [^{99m}Tc]glucarate uptake has the potential to impact cancer patient treatment and management.

[^{99m}Tc]-Glucarate has been described as an imaging agent for acute cerebral injury and myocardial infarction [11–13,19–23] and as a tumor tracer [14–17,22,24], which is not affected by the MDR phenotype [25]. The aim of the present study was to formulate a Glucarate kit and evaluate [^{99m}Tc]glucarate as a potential imaging agent in SCID mice bearing xenograft with MDA-MB-435 breast tumors.

2. Methods

2.1. Kit composition, labeling and quality control

Kit formulation was based on the published method of Babbar et al. [26] with minor changes. The Glucarate kit composition was 12 mg mono potassium glucarate, 0.1 mg $SnCl_2.2H_2O$, sodium bicarbonate, acetic and ascorbic acid. The reagents in the vial were sterile, apyrogenic and lyophilized as a composition kit. The stability of the Glucarate kits was examined over a 6-month period. [^{99m}Tc]Glucarate was obtained after adding up to 1110 MBq in 1 ml of $^{99m}TcO_4^-$ solution (370–1110 MBq, pH 8), obtained from a ^{99}Mo/^{99m}Tc generator (Tecnonuclear), to the Glucarate kit. The kit was shaken for 1 min and kept at room temperature for 20 min, after which time the pH was adjusted to 7–7.5 adding 200 μl of HCl (0.1N). Radiochemical purity was assayed by the following methods: Whatman No. 1 paper as stationary phase and acetone and NaCl 0.9% as mobile phases (systems I and II). In the first system, [^{99m}Tc]glucarate and ^{99m}Tc-colloid remained at the origin, while ^{99m}Tc-pertechnetate moved near the solvent front. In the second system, using saline as solvent, ^{99m}Tc-colloid remained at the origin while [^{99m}Tc]glucarate and ^{99m}Tc-pertechnetate migrated near the solvent front.

2.2. Cell lines and cell culture

Human breast carcinoma cell line MDA-MB-435 was received from the American Type Tissue Culture (Manassas, VA, USA). The cells were maintained as monolayer cultures in RPMI 1640 custom media (Invitrogen, St. Louis, MO, USA) with 10% fetal bovine serum and 0.06 mg/ml gentamycin. Cultures were maintained at 37°C in a 5% CO_2 humidified incubator.

2.3. Animal studies

All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by the Institutional Animal Care and Use Committees of both the Harry S. Truman Memorial Veterans Hospital and the University of Missouri. Female 4–6-week-old SCID (ICR SCID) mice (Taconic, Hudson, NY, USA) were inoculated subcutaneously in the shoulder with 5×10^6 cultured MDA-MB-435 human breast carcinoma cells. The biodistribution, single photon emission tomography (SPECT)/X-ray computed tomography (CT) imaging of the [^{99m}Tc]glucarate and micro-magnetic resonance imaging (MRI) were performed in the MDA-MB-435 breast carcinoma bearing mice when the tumors reached approximately 0.5 g.

2.4. In vivo biodistribution studies

The pharmacokinetics of [^{99m}Tc]glucarate were determined in female SCID mice bearing MDA-MB-435 human breast carcinoma tumors. Approximately 0.185–1.48 MBq (5–40 μCi) of [^{99m}Tc]glucarate was injected into the mouse via the tail vein. Mice (n=4) were euthanized at 0.5 1, 2, 4 and 24 h post injection, with tumors and organs of interest harvested and weighed and radioactivity quantitated in a Wallac Wizard 3 gamma counter. Uptake of radioactivity in the tumor and normal tissues and organs was expressed as a percentage of the radioactive injected dose per gram (% ID/g) or percentage of radioactive injected dose (% ID).

2.5. Micro-MRI studies

Micro-MRI studies were performed on tumor bearing mice prior to injection with [^{99m}Tc]glucarate. Diffusion-, T_1- and T_2-weighted studies were carried out on live mice anesthetized with isoflurane using a 7T 210 mm horizontal bore MRI