Biomaterials 67 (2015) 160–168

Contents lists available at ScienceDirect

Biomaterials

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

A multimodal nano agent for image-guided cancer surgery

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ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form
6 July 2015
Accepted 10 July 2015
Available online 14 July 2015

Keywords:
Image-guided surgery
Liposomes
Multimodality imaging
Computed tomography
Nearinfrared fluorescence
Cancer

ABSTRACT

Intraoperative imaging technologies including computed tomography and fluorescence optical imaging are becoming routine tools in the cancer surgery operating room. They constitute an enabling platform for high performance surgical resections that assure local control while minimizing morbidity. New contrast agents that can increase the sensitivity and visualization power of existing intraoperative imaging techniques will further enhance their clinical benefit. We report here the development, detection and visualization of a dual-modality computed tomography and near-infrared fluorescence nano liposomal agent (CF800) in multiple preclinical animal models of cancer. We describe the successful application of this agent for combined preoperative computed tomography based three-dimensional surgical planning and intraoperative target mapping (>200 Hounsfield Units enhancement), as well as near-infrared fluorescence guided resection (>5-fold tumor-to-background ratio). These results strongly support the clinical advancement of this agent for image-guided surgery with potential to improve lesion localization, margin delineation and metastatic lymph node detection.

1. Introduction

Surgery is the primary treatment modality for removal and debulking of localized tumors [1]. Surgical performance strongly affects survival [2–4]. Innovations such as the integration of imaging technologies into the operating room occurred in response to effective screening programs and the challenge of resecting small tumors. The growing use of intraoperative computed tomography (CT) [5,6] and optical imaging [7–9] enabled the advancement of high precision tissue conserving surgery. This motivated our development of a multi-modal surgical guidance imaging agent to further enhance the sensitivity of preoperative and intraoperative target localization and intraoperative navigation.

Increasing the performance of lesion visualization and mapping can positively impact surgery in a number of different ways. Surgical removal of head and neck (H&N) cancers is a good example of resection tasks that occur in close proximity to critical structures, which can potentially limit surgical performance even for the experienced surgeon. Clinicians must contend with complex 3D anatomical structures that are subject to variations across the patient population and morphological changes due to disease pathology or prior surgery. This need for precise surgical guidance that accounts for intraoperative anatomical deformation and tissue excision resulted in the clinical adoption of imaging systems such as intraoperative cone beam CT (CBCT). In fact, the relevance of CBCT image-guidance to improve target localization and avoidance of critical structures has been previously demonstrated by our group in a prospective clinical trial [6]. Lung cancer surgery, on the

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http://dx.doi.org/10.1016/j.biomaterials.2015.07.010

other hand, poses a different challenge in that thoracic surgeons are required to resect increasingly small lung nodules that are often non-visible and non-palpable. This is a result of effective screening programs of high risk groups which allowed for earlier diagnosis of the disease in a significant number of patients [10]. There is, therefore, an unmet need for novel intraoperative visualization strategies that can effectively aid the surgeon to accurately localize the resection target and perform appropriate tissue-conserving surgeries for these small tumors. In fact, Suzuki et al. [11] reported that frequent conversion to thoracotomy (54%) was needed due to failure to localize nodules (46%). The current standard procedure at our institution for intraoperative localization of small sized lung nodules involves CT-guided micro-coil placement performed prior to video assisted thoracic surgery (VATS), intraoperative visualization of the micro-coil under fluoroscopy and its resection together with the pulmonary nodule. Limitations associated with this procedure include frequent pneumothorax following fiducial placement and radiation exposure for both the patient and surgical staff during repeated intraoperative fluoroscopy. A viable strategy would consist in using our dual-modality imaging agent for CT-based localization of tumor and fluorescence-guided resection.

A number of near-infrared (NIR) fluorescence probes have been explored for intraoperative image-guidance in preclinical models [12–15] and in exploratory clinical trials [16–21] with varying degrees of success. Of these, two agents [16–18,21] have demonstrated capability to support multimodality imaging and have been employed in clinical trials, for I^{124} positron emission tomography (PET) and Tc^{99m} single photon emission computed tomography (SPECT) based preoperative planning imaging, respectively, in addition to NIR fluorescence surgical guidance. However, nuclear imaging with PET and SPECT lack the high spatial resolution offered by CT (multi-mm versus sub-mm voxel size). High spatial resolution imaging is required for accurate tumor margin delineation and localization of small lesions as well as potential micrometastases. Here we report, for the first time, the development, preclinical characterization and performance assessment of a CT and NIR fluorescence liposome-based dual-modality imaging agent (CF800). CF800 is an injectable nanosystem that co-encapsulates multiple imaging molecules with relative concentrations engineered to match the clinical imaging requirements for CT and NIR fluorescence imaging. This agent is composed of regulatory approved components and it is specifically designed for longitudinal preoperative and intraoperative tumor and malignant lymph node visualization and mapping. Effective assembly of proven technologies and their integration into existing clinical practice has the potential to achieve accelerated clinical impact.

2. Materials and methods

2.1. Liposome preparation

The liposome bilayer is composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, CordenPharma, Liestal, Switzerland), cholesterol (Northern Lipids Inc., Vancouver, BC, Canada) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol)2000] (PEG_{2000}DSPE, CordenPharma, Liestal, Switzerland) in a percent mole ratio of 55:40:5. The liposome preparation procedure is modified from a previously published protocol [22,23]. Specifically, the lipid mixture was first dissolved in ethanol and then hydrated for 4 h at 70 °C in a solution of 300 μg of ICG (IR-125, Acros Organics, Geel, Belgium) dissolved in Omnipaque350® (GE Healthcare, Milwaukee, Wisconsin). The resulting liposome solution was extruded at pressures between 100 and 400 psi using the LIPEX™ Extruder (Northern Lipids Inc., Vancouver, Canada). Purification was performed using a Sephadex G-25 column. The final liposome sample was kept at room temperature protected from light until use.

2.2. Physico-chemical characterization of liposomes

The size of the liposome samples was measured using dynamic light scattering (DLS) (90Plus, Brookhaven, Holtsville, New York). The iodine and ICG concentrations were determined using an absorbance assay at a wavelength of 245 nm for iodine and 785 nm for ICG (Cary 50 UV/VIS Spectrophotometer, Varian Inc., Palo Alto, California). The lipid components of the liposome sample was analyzed using ultra performance liquid chromatography (UPLC, ACQUITY, Waters, Milford, Massachusetts) equipped with an evaporative light scattering detector (ELSD). The *in vitro* fluorescence measurements were performed using a 2D fluorescence imager (excitation/emission: 735/820 nm, CRI Maestro, Perkin Elmer, Waltham, Massachusetts).

2.3. Animal models

All preclinical investigations were approved by the University Health Network Animal Care Committee. Two disseminated models of human cancer in female SCID mice were employed: 1) SKOV-3/Luc ovarian adenocarcinoma (purchased from Cell Biolabs Inc., San Diego, California) inoculated intraperitoneally (3×10^6 cells in 100 μL), and 2) LM2-4H2N/Luc (a metastatic variant of the breast cancer cell line MDA-MB-231 [24]) injected into the right lower mammary fat pad (4×10^6 cells in 50 μL). Bioluminescence imaging (IVIS Imaging System, Perkin Elmer, Waltham, Massachusetts) at 10 min post *i.p.* injection of 150 mg/kg D-luciferin was used to monitor tumor growth and metastasis formation in all mice. Rabbit models of VX-2 buccal mucosa and lung cancer were also employed in this study. For the buccal mucosa tumor model, 300 μL of a VX-2 carcinoma cell suspension (5×10^6 cells/mL) obtained from propagation rabbits [25] was injected into the buccinator muscle of New Zealand White rabbits (Charles River, Wilmington, Massachusetts). Tumors were formed at the site of VX-2 cell injection and all rabbits presented with at least one cervical lymph node metastasis at two weeks post inoculation. The VX-2 rabbit lung tumor model was established according to previously reported methods [26].

2.4. In vivo and ex vivo imaging

Each mouse (20–25 g) received 200 μL of the liposome formulation *i.v.* (0.58 ± 0.05 mg/g of iodine and 1.2 ± 0.3 μg/g of ICG, diameter = 86 ± 4 nm) and each rabbit (2.6–3.0 kg) received 20 mL of the liposome formulation *i.v.* (0.39 ± 0.02 g/kg of iodine and 1.0 ± 0.3 mg/kg of ICG, diameter = 90 ± 2 nm). CT imaging (Locus Ultra, GE Healthcare, Milwaukee, Wisconsin) was performed pre and post liposome administration (80 kVp, 50 mA). It is estimated that the total whole body dose per mouse per scan is less than 10 cGy [27], this is ~1% of the LD50/30 (radiation dose lethal for 50% of mice within 30 days post-exposure) reported for mice [28]. For the mouse study, at 48 h *p.i.*, animals were sacrificed and their peritoneal cavities were exposed for post-mortem CT and 2D NIR fluorescence imaging to evaluate the co-localization of the CT and fluorescence signals. For the rabbit study, image-guided surgical removal of the primary tumor and involved lymph nodes was performed at 4–7 days post-liposome administration. Real-time fluorescence imaging (overlaid with white light) of the surgical site containing the tumor and malignant lymph nodes was performed using the PINPOINT® system (808 ± 5 nm, Novadaq, Mississauga, Canada).