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A kit to prepare ^{111}In-DTPA-trastuzumab (Herceptin) Fab fragments injection under GMP conditions for imaging or radioimmunoguided surgery of HER2-positive breast cancer☆

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Abstract

Introduction: The human epidermal growth factor receptor-2 (HER2) gene is amplified in 25% of invasive breast cancers, and receptor overexpression has been noted in up to 60% of early stages of the disease [ductal carcinoma in situ (DCIS)]. Preclinical studies have revealed high tumor/blood ratios (>27:1) for ^{111}In-labeled Fab fragments of the HER2 monoclonal antibody, trastuzumab (Herceptin) (^{111}In-DTPA-trastuzumab Fab) at 72 h pi in athymic mice bearing subcutaneous human breast cancer xenografts. Our aim in this study was to formulate a kit for preparation of ^{111}In-DTPA-trastuzumab Fab injection under good manufacturing practice (GMP) conditions suitable for human administration in a Phase I clinical trial of imaging and radioimmunoguided surgery (RIGS) of HER2-positive breast cancer.
Methods: Fab fragments were produced by digestion of trastuzumab IgG (Herceptin) with immobilized papain for 20 h at 37°C. Fab fragments were purified by ultrafiltration, then reacted with a 10-fold molar excess of diethylenetriaminepentaacetic acid (DTPA) dianhydride. DTPA-Fab fragments were purified, then sterilized by filtration into unit dose glass vials (kits). Kits were tested against specifications for volume (0.9–1.1 ml), protein concentration (0.45–0.55 mg/ml), pH (5.5–6.5), DTPA substitution (0.5–4.0 mol DTPA/mol Fab), appearance (clear, colorless and particle free), labeling efficiency (≥85%), and sterility and apyrogenicity (USP XXXII). Immunoreactivity of ^{111}In-DTPA-trastuzumab Fab towards HER2 was measured by saturation radioligand binding assays using SKBR-3 human breast cancer cells (specifications: K_a=0.6–9.6×10^7 L/mol; B_{max}=0.6–10.4×10^6 sites/cell). ^{111}In-DTPA-trastuzumab Fab injection was prepared by adding 80–100 MBq of $^{111}InCl_3$ to a single kit vial and incubating for 30 min at room temperature. ^{111}In-DTPA-trastuzumab Fab was assayed for the amount of radioactivity and tested for pH, radiochemical purity (RCP), appearance and sterility.
Results: Pure and homogeneous Fab fragments were produced. Eleven lots of kits met established quality specifications. The labeling efficiency with ^{111}In was 90.6±2.2%. ^{111}In-DTPA-trastuzumab Fab bound specifically to HER2 on SKBR-3 cells (K_a=4.8±2.5×10^7 L/mol and B_{max}=1.6±0.8×10^6 sites/cell). Thirteen lots of ^{111}In-DTPA-trastuzumab injection met all established specifications. Kits were stable for 90 days and ^{111}In-DTPA-trastuzumab Fab injection was stable for 24 h stored at 4°C.
Conclusions: A kit was formulated under GMP conditions for the preparation of ^{111}In-DTPA-trastuzumab Fab injection suitable for human administration. The kits were approved by Health Canada.

Keywords: Breast cancer; Trastuzumab Fab; ^{111}In; kit; Good manufacturing practices (GMP); Radioimmunoguided surgery (RIGS); Imaging

1. Introduction

The human epidermal growth factor receptor-2 (HER2) is overexpressed due to gene amplification in about 25% of invasive breast cancers [1]. In early stages of breast cancer, i.e., ductal carcinoma in situ (DCIS), HER2 overexpression may be present in up to 60% of patients [2]. HER2 positivity

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is associated with estrogen receptor negativity and with a lack of response to hormonal therapy as well as to chemotherapy and with a poor outcome [3,4]. Thus HER2 overexpression represents an attractive target for development of molecular imaging agents for detection of invasive breast tumors and their metastases as well as for identifying early stages of the disease. Imaging may yield information on tumor dissemination as well as reveal the HER2 phenotype of lesions in situ, which would be valuable to select patients for treatment with HER2-targeted therapies [5,6]. In theory, these probes may also be useful for delineating the margins of primary HER2-positive tumors in the breast to aid in surgical resection [radioimmunoguided surgery (RIGS)]. In RIGS, patients are administered a radiopharmaceutical prior to surgery which targets and "radioactively marks" the distribution of tumor cells for intraoperative detection using a handheld γ-detecting probe. Accurate intraoperative tumor delineation facilitates complete resection, with the aim of improving the accuracy of surgical excision to prevent recurrence and optimize cosmesis [7,8].

HER2 overexpression has been imaged preclinically in human breast cancer xenograft mouse models or clinically in breast cancer patients using radiolabeled intact IgG monoclonal antibodies (mAbs) [9–12], Fab fragments [13–15] or novel recombinant antibody forms such as scFv, diabodies or minibodies [16–18]. HER2-binding affibodies labeled with ^{99m}Tc, ^{111}In or ^{18}F have also shown promise for imaging HER2 [19,20]. For example, Orlova et al. [21] reported that ^{124}I-labeled ZHER2:342 affibodies exhibited lower tumor uptake than ^{124}I-labeled intact IgG anti-HER2 mAb trastuzumab (Herceptin, Hoffman La Roche) [0.4 vs. 12 percent injected dose/gram (% ID/g)] but demonstrated much higher tumor/blood (T/B) ratios (27:1 vs. 1.3:1) at 72 h postinjection (pi) in athymic mice bearing subcutaneous HER2-positive NCI-N87 gastric adenocarcinoma xenografts.

High T/B and tumor/normal tissue ratios are desirable for tumor imaging as well as RIGS and could be obtained using Fab fragments, since these are rapidly eliminated from the blood and most normal tissues (except kidneys) [22]. In previous work, we compared the tumor and normal tissue distribution and imaging properties of trastuzumab Fab fragments labeled with ^{99m}Tc or ^{111}In, in athymic mice implanted subcutaneously with HER2-positive BT-474 human breast cancer xenografts [14,15]. Tumors were visualized at 6 or 24 h pi of ^{99m}Tc-HYNIC-trastuzumab Fab fragments and tumor uptake was 10 %ID/g with T/B ratios of 3:1 [15]. However, much higher T/B ratios were achieved with ^{111}In-DTPA-trastuzumab Fab at 72 h pi (>25:1), while tumor uptake at this time point (8 % ID/g) was similar to that for ^{99m}Tc-HYNIC-trastuzumab Fab [14]. These results suggested that ^{111}In-DTPA-trastuzumab Fab fragments may offer advantages for imaging and RIGS of HER2-overexpressing breast cancer in humans by permitting delayed imaging and intraoperative detection of tumors when blood radioactivity levels are lower and T/B ratios higher.

In order to translate these encouraging preclinical findings for ^{111}In-DTPA-trastuzumab Fab fragments to imaging or RIGS in humans with HER2-amplified breast cancer, we designed a kit under good manufacturing practices (GMP) conditions to prepare the radiopharmaceutical in a quality suitable for human administration. The formulation of these kits, establishment of specifications for their quality, as well as the results of quality control testing are described in this report. These kits were approved by Health Canada for the preparation of ^{111}In-DTPA-trastuzumab Fab injection for a Phase I clinical trial of imaging and RIGS of HER2-positive breast cancer conducted at Sunnybrook Health Sciences Centre in Toronto, Ontario, Canada (Protocol No. 261-2006).

2. Materials and methods

2.1. Raw materials

Trastuzumab IgG (Herceptin; Hoffman La Roche, Mississauga, ON, Canada) was reconstituted to 21 mg/ml with Sterile Water for Injection, USP following the manufacturer's directions. Sodium acetate dihydrate, USP ($NaCH_3COO{\cdot}2H_2O$) and sodium bicarbonate, USP ($NaHCO_3$) were obtained from EMD Chemicals, Inc. (Gibbstown, NJ, USA). Sodium phosphate monobasic monohydrate, USP ($NaH_2PO_4{\cdot}H_2O$) and immobilized papain containing 250 μg papain/ml of agarose gel (Pierce Chemical Co., Product No. 20341) were obtained from Fisher Scientific Co. (Ottawa, ON, Canada). Chloroform ($CHCl_3$) was ultrapure reagent grade (>99.9%; Sigma-Aldrich, St. Louis, MO, USA). L-Cysteine (purity >99.5%) and diethylenetriaminepentaacetic acid (DTPA) dianhydride (purity >95%) were obtained from Sigma-Aldrich. All other chemicals and reagents were purchased in analytical ACS grade with a minimum purity of 95%. Sterile, apyrogenic type 1 glass multidose vials (5 or 10 ml) with a grey butyl rubber septum and aluminum seal were obtained from Hollister-Stier Laboratories, Inc. (Spokane, WA, USA). Radiochemical quality $^{111}InCl_3$ (>3.7 GBq/ml; <0.1% ^{114m}In and ^{65}Zn) was purchased from MDS-Nordion, Inc. (Kanata, ON, Canada). Certificates of analysis assuring the specified purity were obtained from the suppliers of all chemicals, and identity tests were performed in-house as previously reported [23].

2.2. Pharmaceutical buffers

IgG digestion buffer was prepared and consisted of 20 mM $NaH_2PO_4{\cdot}H_2O$ and 10 mM disodium ethylenediaminetetraacetic acid (Na_2EDTA) in Sterile Water for Irrigation, USP (pH 7.0). In addition, 50 mM $NaHCO_3$ buffer (pH 7.5) and 1 M $NaCH_3COO$ buffer (pH 6.0) were prepared as reported [23]. Trace metals were removed from all buffers by passing them through a 20 ml column of Chelex-100 cation exchange resin (BioRad, Mississauga,