Clinica Chimica Acta 412 (2011) 1572–1577

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

A rapid and sensitive chemiluminescence immunoassay based on magnetic particles for squamous cell carcinoma antigen in human serum

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ARTICLE INFO

Article history:
Received 23 January 2011
Received in revised form 1 May 2011
Accepted 3 May 2011
Available online 12 May 2011

Keywords:
Chemiluminescence immunoassay
Squamous cell carcinoma antigen
Magnetic particles

ABSTRACT

Background: Because squamous cell carcinoma antigen (SCCa) quantification has demonstrated strong clinical potential, we describe a rapid and highly sensitive magnetic particle-based chemiluminescence immunoassay (CLIA) technique for assaying SCCa in serum.
Methods: Fluorescein isothiocyanate (FITC) and N-(aminobutyl)-N-(ethylisoluminol) (ABEI) were used to label 2 different monoclonal antibodies of anti-SCCa. Both of the labeled antibodies combined with SCCa to form a sandwiched immunoreaction that was monitored by chemiluminescence (CL) detection. The magnetic particles (MPs) that were coated with anti-FITC antibody served as both the solid phase and the separator. The relevant variables involved in the CLIA signals were optimized and the parameters of the proposed method were evaluated.
Results: The method was linear to 20 ng/ml SCCa with a detection limit of 0.02 ng/ml. The intra-assay imprecision results [mean (CV)] were 1.12 ng/ml (3.81%), 2.58 ng/ml (2.53%) and [6.56 ng/ml (2.24%)]; the inter-assay imprecision results were [1.18 ng/ml (5.26%)], [2.49 ng/ml (4.75%)] and [6.61 ng/ml (4.29%)]. The average recoveries were between 97% and 104%. The relationship between the concentration of diluted SCCa and the dilution ratios gave a linear correlation coefficient of 0.9995. A correlation analysis against an established automated assay generated a slope of 0.9929 and an intercept of 0.0039 ng/ml ($r = 0.9964$).
Conclusions: The proposed method demonstrates an acceptable performance for quantifying serum SCCa and is suitable for the fabrication of a commercial kit with application in the automated CL analyzer.

1. Introduction

Squamous cell carcinoma antigen (SCCa), a glycoprotein with isoforms ranging from 45 to 55 kDa [1], was first described as a tumor-associated antigen by Kato and Torigoe [2]. It is physiologically expressed in the skin and other squamous epithelial cells [3,4] and has been proven helpful in the diagnosis of lung cancer, especially squamous cell carcinoma [5–7]. High levels of SCCa have been detected in the tissues of the head and neck, the liver and other epithelial carcinomas [8,9]. It has also been reported to be over-expressed in the serum of patients with various carcinomas of the uterine cervix, oral cavity, lung, skin, head and neck, esophagus, anal canal and vulva [7,10–17]. For example, the level of serum SCCa increased by 72% in patients with cervical carcinomas and increased by 94% in patients with lung squamous cell carcinomas [5,10]. The detection of the SCCa level of cervical squamous cell carcinomas can be used for the assessment of lymph node metastasis and for guiding the operative choice. Preoperative enhanced serum SCCa levels in squamous cell carcinoma of the cervix and lung are usually accompanied by a higher stage and a poorer prognosis [18,19]. Elevated levels of serum SCCa and lymph node metastasis are independent predictors in untreated cervical carcinoma patients [20]. The SCCa serum levels after radiotherapy may influence whether the cervical squamous cell carcinoma recurs [21]. In the treatment of head and neck tumors, a high level of SCCa indicates the incomplete surgical resection of the tumor or a resistance to treatment. Furthermore, SCCa levels generally increase 3 months prior to the recurrence of clinical symptoms [22].

Enzyme-linked immunosorbent assay (ELISA) [23,24], radioimmunoassay (RIA) [25] and chemiluminescence enzyme immunoassay (CLEIA) are often used to detect the total SCCa in serum. However, the ELISA method has limitations such as poor reproducibility, low sensitivity and narrow linear range. Although RIA methods are considered accurate and reliable, the drawbacks of radioisotopes, waste disposal problems, the need for a specialized laboratory, high cost and a short half-life have induced an intensive search for alternative methods. During recent years, chemiluminescence enzyme

Abbreviations: CLIA, chemiluminescence immunoassay; SCCa, squamous cell carcinoma antigen; FITC, fluorescein isothiocyanate; ABEI, N-(aminobutyl)-N-(ethylisoluminol); CL, chemiluminescence; MPs, magnetic particles; BSA, bovine serum albumin; EDC, 1-ethyl-3-3(3-dimethylaminopropyl) carbodiimide hydrochloride; NHS, N-hydroxysuccinimide; PBS, phosphate buffered saline; CLEIA, chemiluminescence enzyme immunoassay; RLU, relative light unit; CR, cross-reactivity; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen; CYFRA21-1, cytokeratin 19 fragment.

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doi:10.1016/j.cca.2011.05.005

immunoassay (CLEIA) and chemiluminescence immunoassay (CLIA) have been used in clinical diagnoses because of the advantages of no radioactive pollution, an acceptable sensitivity and a wide dynamic range [26,27]. CLEIA is an indirect luminescence measurement which uses an enzyme-labeled antigen or antibody and enzyme-catalyzing chemiluminescent reagents to emit light. Compared with CLEIA, CLIA is a simpler luminescence measurement using chemiluminescent reagents, such as aeridinium ester and N-(aminobutyl)-N-(ethylisoluminol) (ABEI), to directly label the antigen or antibody, without the participation of an enzyme.

Here, we describe a simple CLIA for SCCa in which the advantages of a monoclonal antibody labeled with ABEI are combined with a simplified separation procedure based on the use of magnetic particles (MPs). The chemiluminescence (CL) signal produced by the emission of photons from ABEI was directly proportional to the amount of analyte present in the sample solution. The experimental conditions, including the dilution ratio of the ABEI-labeled anti-SCCa antibody and the fluorescent isothiocyanate (FITC)- labeled anti-SCCa antibody, the volume of the magnetic particles and the substrate and the incubation time were examined and optimized. In addition, the methodology parameters including precision, sensitivity and specificity, for example, were also evaluated. The results showed that the present method is credible and is suitable for the fabrication of a commercial kit with application in the automated CL analyzer.

2. Materials and methods

2.1. Chemicals, immunoreagents and apparatus

SCCa stock solution, FITC, N-hydroxysuccinimide (NHS), ABEI and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) from Sigma-Aldrich (St. Louis, MO). The immunomagnetic particles (5.0 mg/ml) coated with goat anti-mouse FITC polyclonal antibody were from Adaltis, Inc. (Rome, Italy). Mouse anti-human SCCa monoclonal antibodies of 2 different clones were from Fitzgerald (Concord, MA). The chemiluminescent substrate (sodium hydroxide and H_2O_2), bovine serum albumin (BSA) and dimethyl formamide were purchased from Beijing JingKeHongDa Biotech Co. Ltd. (Beijing, China). The biuret-phenol reagent was from Sigma. The incubation and shaking procedures were carried out in a thermostated container (FYL-YS, China) and shaker (ZXWL-100, China). The immunoassay procedures were performed using a Maglumi 2000 Plus automatic analyzer from New Industries Biomedical Engineering Co. Ltd. (Shenzhen, China). The commercially-available SCCa kit and the instrument for methodology comparison were provided by Abbott China Co.

2.2. Buffers, calibrators and samples

The washing buffer was 0.05 mol/l TRIS solution with 0.05% Tween-20 (PBST). The 20% bovine serum and the solution of 0.1 mol/l Tris–HCl buffer (pH 7.6) containing 0.5% BSA and 0.5% glycerol were used as dilution buffers for the ABEI-labeled anti-SCCa antibody and the FITC-labeled anti-SCCa antibody, respectively. A carbonate buffer solution (pH 9.3) and phosphate-buffered saline (PBS, pH 7.4) were used for FITC and ABEI to label anti-SCCa antibodies. Calibrators were prepared by diluting SCCa stock with BSA/PBS solution (0.008 mol/l Na_2HPO_4, 0.003 mol/l KH_2PO_4, 0.150 mol/l NaCl, 10 g/l BSA, pH 7.4) to target values of 0.0, 2.5, 5.0, 10.0, 15.0 and 20.0 ng/ml, which were assigned to S_0, S_1, S_2, S_3, S_4 and S_5 respectively. Aliquots (100 μl) were lyophilized and stored at −70 °C. The human sera from local hospitals were collected and stored at −20 °C until analysis.

2.3. FITC-labeled anti-SCCa antibody

The FITC-labeled anti-SCCa antibody was prepared according to reference [28], but with slight modifications. FITC was dissolved with a small amount of dimethyl formamide and then slowly added into 0.1 mol/l $NaHCO_3$ buffer (pH 9.3) while stirring to obtain the FITC solution (4 mg/ml). The anti-SCCa antibody was put into a dialysis bag

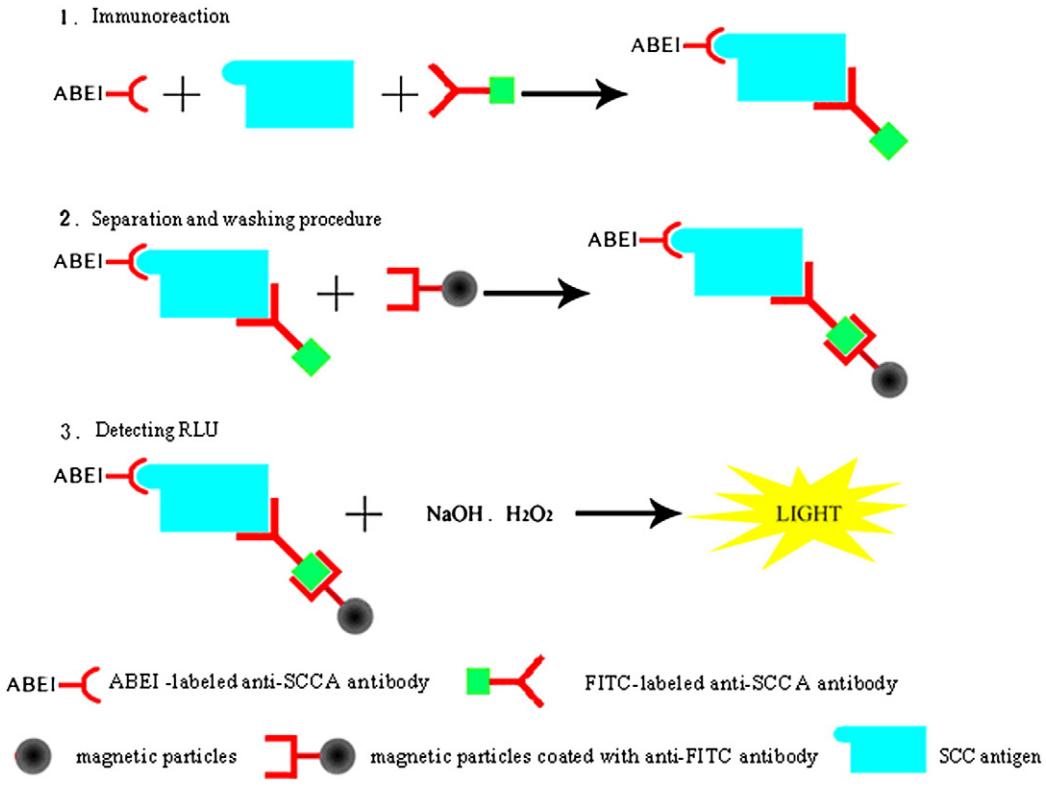

Fig. 1. Schematic illustration of the proposed chemiluminescence immunoassay.