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# A new approach for pharmacokinetics of single-dose cetuximab in rhesus monkeys by surface plasmon resonance biosensor

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ABSTRACT

A novel assay method has been developed and validated, using surface plasmon resonance (SPR), for quantitation of cetuximab (C225) in monkey serum. By injecting non-labeled antibody samples onto a biosensor surface on which epidermal growth factor receptor (EGFR) was immobilized, the concentration of C225 can be accurately measured. This assay has a range of reliable response from 0.05 to 50 μg/ml C225 in monkey serum, which was well fitted with a sigmoidal model. The immobilized EGFR was found to be stable for at least 100 regeneration cycles at room temperature. Intra- and inter-assay CVs ranged from 3.20% to 8.89% and from 5.93% to 11.11%, accuracy from 92% to 107.52% and from 90% to 106.88%, respectively. Matrices such as 50% human serum, 50% Sprague Dawley rat serum, chimeric recombinant anti-CD20 monoclonal antibody, human γ-globulin and chimeric recombinant her2 antibody did not interfere with C225 analysis on the sensor surface. This is the first report on the quantitation of C225 in monkey serum by an optical biosensor technology. This method was used to characterize the pharmacokinetics of C225 in rhesus monkeys. After a single-dose of intravenous infusion administration of 7.5, 24 and 75 μg/kg, average $C_{max}$ ranged from 168 ± 28 to 1624 ± 113 μg/ml, and $AUC_{0-\infty}$ ranged from 15,739 ± 1059 to 295,017 ± 44,533 μg h/ml. C225 elimination followed a bi-exponential profile with $t_{1/2}$ ranging from 2.7 ± 0.7 to 6.7 ± 0.1 h. It was non-linear serum pharmacokinetics of C225 across the investigated dosage range in monkeys (7.5–75 mg/kg).



## 1. Introduction

Epidermal growth factor receptor (EGFR) is a protein tyrosine kinase which plays a crucial role in signal transduction pathways regulating key cellular functions such as survival and proliferation. Among the recent advances in the molecular targeted therapy of cancer, the applications centered on EGFR are currently the most promising and the most advanced at clinical level [1]. Considering the set of therapeutic tools targeting EGFR [2], there are at present two well-identified emerging categories of drugs with monoclonal antibodies (Mabs), on one hand, tyrosine kinase inhibitors (TKIs) and on the other hand, EGFR inhibitors. Cetuximab (C225) is a Mab (IgG1) directed at the extracellular domain of the receptor, and a novel biologic agent that has been shown both in vitro studies and in vivo animal xenograft models to have profound synergy when combined with either platinum drugs or with other chemotherapeutic agents or radiation therapy [3,4]. Cetuximab, a chimerized monoclonal antibody, was developed to target the EGFR. Cetuximab binds to the extracellular domain of the EGFR thereby preventing ligand activation of EGFR [5]. The resultant inhibition of EGFR signaling can lead to cell cycle arrest, cell death via apoptosis, and inhibition of cell invasion and angiogenesis.

Overexpression of EGFR mRNA and/or protein has been documented in a number of malignancies, including ovarian cancer. Approximately 35% to 70% of ovarian cancers expressed EGFR mRNA analyzed via reverse transcriptase–polymerase chain reaction and radioligand binding assays and 98% demonstrated EGFR protein expression by Western blot analysis [6–8]. High EGFR expression in ovarian cancer specimens has been associated with advanced stage, an aggressive phenotype, and poor clinical outcome [6,7,9,10]. In addition, high EGFR expression has been associated with chemotherapy resistance in human cancer cell lines. EGFR expression as well as the expression of EGFR-related proteins has been shown to become more intense and diffuse in tumor specimens obtained after treatment with cisplatin compared to the staining in matched pretreatment tumor specimens [11]. Therefore, the use of an EGFR inhibitor such as cetuximab to disrupt the EGFR signaling pathway could potentially inhibit the emergence of chemotherapy resistance.

In preclinical studies, cetuximab has been found to repress the growth of cultured A431 tumor cell lines and xenografts that express high levels of EGFR [12,13]. Cetuximab has also been

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shown to enhance the effects of a variety of chemotherapeutic agents, including platinum compounds, in a variety of human tumor cell lines that express the EGFR, including ovarian cancer cell lines [9,14,15]. These intriguing preclinical findings have been supported by the results of clinical trials that revealed that the addition of cetuximab plus cisplatin in patients with platinum resistant squamous cell carcinoma of the head and neck resulted in objective responses [16]. Furthermore, phase III clinical trials demonstrated that combination of cetuximab and chemotherapy yielded superior response rates and in some cases improved survival in patients with head and neck cancer and colorectal cancer [17,18].

Some reports on clinical pharmacokinetic study showed that serum concentrations of cetuximab were measured using ELISA [19–22]. However, there is no report in literature on the preclinical pharmacokinetic data.

Radioimmunoassay and ELSA method are traditionally used for quantitative analyses of biotechnology derived drugs in biomatrics. Biomolecular interaction analysis (BIA) from Biacore uses the optical phenomenon of surface plasmon resonance (SPR) to monitor biomolecular interactions in real time without labeling. SPR measures change in refractive index of the solution close to the senor surface, resulting from changes in the mass concentration of molecules in the solution [23]. The Biacore system has some advantages in immunoassays over the conventional ELISA or RI methods. First, SPR technology can be used to measure complex formation without labeling the reactants. Second, complex formation can be monitored in real time, providing detailed information about the reaction kinetics, and equilibrium dissociation constants (affinities). Third, crude samples may be analyzed without sample preparation. Nowadays, researches using Biacore focused on thermodynamics, kinetics and affinity studies. To concentration analysis, there are some reports on determination of substance residues in food or environments [24–27] and few reports on drug quantitation in biomatrics for pharmacokinetic studies using Biacore. Kikuchi et al. [28] first reported Biacore application for pharmacokinetic study. However, there was no validation of the method. In addition, sensitivity of the method was low. The company, which developed cetuximab first, has conducted the SPR measurements for quantitation of C225 [29], but there was no report on the assay method.

Although it has good clinical effect, the price of cetuximab is too high, more than $15,000 per month, to afford for common patients especially patients in developing countries. The company in China manufactured cetuximab by itself and the price is less expensive. In our study, using the Biacore system, we developed a method to measure the concentration of C225 donated by the Chinese company in monkey serum. Here we show the characteristics and advantages of this assay method and its application for pharmacokinetic studies. There is the first report on the quantitation of C225 in monkey serum using Biacore.

## 2. Materials and methods

### 2.1. Materials

- Biacore 3000 biosensor instrument, amine coupling kit, sensor chip CM5 and P-20 Surfacant (BIACORE, Uppsala, Sweden)
- Running buffer–HBS buffer (HEPES buffered saline): 10 mM HEPES with 0.15 M NaCl, 3.4 mM EDTA and 0.05% P-20 surface, pH 7.4
- Sample diluent: HBS containing 1 mg/ml carboxymethyl dextran (Fluka Chemical Corp., Ronkonkoma, NY)
- Regeneration solution: 10 mM HCl + 1 M NaCl
- Pooled normal monkey serum from Laboratory Animal Center of the Academy of Military Medical Sciences
- EGFR (170 kD) was purchased from Sigma Corporation.
- C225 was donated by Huabei Pharmaceutical Factory, Hebei Province, China.

### 2.2. Animal

Rhesus monkeys were supplied by the Laboratory Animal Center of the Academy of Military Medical Sciences. The animals were individually housed in stainless steel cages in a room with controlled temperature ($25 \pm 1^{\circ}$C) and humidity ($55 \pm 5\%$) and a 12 h light/dark cycle.

The animals were fed with standard diet and had free access to water. All procedures involving animals and their care were carried out according to the guidelines of the Institutional Ethical Committee for Care and Use of Laboratory Animal of Academy of Military Medical Sciences in accordance with the governmental guidelines on animal experimentation, National Institutes of Health "Principles of Laboratory Animal Care".

### 2.3. Biacore quantitative assay for C225 in monkey serum

EGFR was selected for C225 analysis and immobilized onto a flow cell of CM5 sensor chip using the amine coupling kit according to the procedure described by the manufacturer. During immobilization, HBS was used as a mobile phase at a flow rate of 10 μl/min. The carboxymethyl dextran matrix of the sensor chip surface was first activated with an injection of 70 μl of the EDC/NHS reagent mixture. Then, 70 μl of EGFR (1 μg/ml in 10 mM NaOAc (pH 5.7)) was injected and allowed to covalently couple to the sensor surface. Finally, the unreacted sites were blocked by injection of 70 μl of 1 M ethanolamine (pH 8.5). The samples were assayed over the immobilized EGFR sensor surface at room temperature. The mobile phase was HBS at a flow rate of 10 μl/min. Biacore response of about 5500 RU was achieved after immobilization. For concentration measurements, an untreated surface was used as the reference cell. During analysis, 10 μl of C225 standard sample and five times diluted serum sample was injected and passed over the reference and the EGFR immobilized surface. Data from the reference flow cell were subtracted to remove the effects of non-specific binding. Regeneration of the sensor surface was achieved by injecting 10 μl of 10 mM HCl plus 1 M NaCl, followed by a return to HBS.

### 2.4. Validation of Biacore quantitative assay for C225 in monkey serum

Validation was based on the FDA guidelines for Bioanalytical Method Validation [29].

#### 2.4.1. Construction of C225 standard curve

A series of six standard samples from 50 to 0.05 μg/ml of C225 in monkey serum were prepared. Before assayed, the standard samples were diluted 1:5 into sample diluent. The relationship between Biacore response and C225 concentration was described by a four-parameter sigmoidal model:

$$R = \frac{R_{\max} + (R_{\min} - R_{\max})}{[1 + (C/\mathrm{EC}_{50})^{r}]} \quad (1)$$

where $R$ is Biacore response, $R_{\max}$ is the estimated maximum of the function, $R_{\min}$ is the estimated minimum of the function, $C$ is C225 concentration, $\mathrm{EC}_{50}$ is the estimated midpoint of the regression line, and $r$ is the slope of the apparent linear region of the curve. A logarithmic calibration plot was then constructed of the normalized binding response versus C225 concentration.