Biosensors and Bioelectronics 95 (2017) 55–59

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Reliable clinical serum analysis with reusable electrochemical sensor: Toward point-of-care measurement of the antipsychotic medication clozapine

Mijeong Kang[a,b], Eunkyoung Kim[a,b], Thomas E. Winkler[b,c], George Banis[b,c], Yi Liu[a,b], Christopher A. Kitchen[d], Deanna L. Kelly[d], Reza Ghodssi[b,c,e], Gregory F. Payne[a,b,*]

[a] Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, MD 20742, United States
[b] Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, United States
[c] MEMS Sensors and Actuators Laboratory (MSAL), University of Maryland, College Park, MD 20742, United States
[d] Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228, United States
[e] Department of Electrical and Computer Engineering, Institute for Systems Research, University of Maryland, College Park, MD 20742, United States

ARTICLE INFO

Keywords:
Therapeutic drug monitoring
Electrochemical sensor
Serum
Clozapine
Graphene
Electrodeposition

ABSTRACT

Clozapine is one of the most promising medications for managing schizophrenia but it is under-utilized because of the challenges of maintaining serum levels in a safe therapeutic range (1–3 μM). Timely measurement of serum clozapine levels has been identified as a barrier to the broader use of clozapine, which is however challenging due to the complexity of serum samples. We demonstrate a robust and reusable electrochemical sensor with graphene-chitosan composite for rapidly measuring serum levels of clozapine. Our electrochemical measurements in clinical serum from clozapine-treated and clozapine-untreated schizophrenia groups are well correlated to centralized laboratory analysis for the readily detected uric acid and for the clozapine which is present at 100-fold lower concentration. The benefits of our electrochemical measurement approach for serum clozapine monitoring are: (i) rapid measurement (≈20 min) without serum pretreatment; (ii) appropriate selectivity and sensitivity (limit of detection 0.7 μM); (iii) reusability of an electrode over several weeks; and (iv) rapid reliability testing to detect common error-causing problems. This simple and rapid electrochemical approach for serum clozapine measurements should provide clinicians with the timely point-of-care information required to adjust dosages and personalize the management of schizophrenia.

1. Introduction

Schizophrenia is a devastating disorder that is poorly understood and difficult to manage. Clozapine (Wenthur and Lindsley, 2013) is one of the most promising medications for treating refractory schizophrenia and controlling violent behaviors and risks of suicide (Fakra and Azorin, 2012). Many experts believe that a broader use of clozapine would benefit both individuals and the larger society, but clinicians are often hesitant to prescribe clozapine because of its adverse side effects (Fakra and Azorin, 2012; Freudenreich et al., 2013; Gee et al., 2014; Kelly et al., 2007; Nielsen et al., 2012; Warnez and Alessi- Severini, 2014). In fact, clozapine's side effect risks led to its removal from the market, but it was later re-introduced because of its unique therapeutic benefits (Warnez and Alessi- Severini, 2014; Wenthur and Lindsley, 2013). As illustrated in Fig. 1**a**, the challenge to clinicians is that serum clozapine levels cannot be adequately controlled by dosage because of variabilities in drug metabolism (Couchman et al., 2013; Rajkumar et al., 2013; Rostami- Hodjegan et al., 2004). A recent survey indicated that clozapine would be more broadly prescribed if serum levels could be rapidly measured at the point-of-care (POC) to provide clinicians with the timely information required to adjust dosages (Kelly et al., 2015).

Currently, clozapine is analyzed in centralized laboratories, using instrument-intensive methods (e.g., high-performance liquid chromatography and mass spectrometry) that require several days turnaround. Since clozapine has intrinsic electrochemical activity, a simple electrochemical method could possibly provide the timely POC analysis as illustrated in Fig. 1**b**. In fact, several groups have begun developing electrochemical methods and significant progress has been made to achieve the high sensitivities required for clinical analysis in the relevant therapeutic range (1–3 μM; 1000-fold lower than serum glucose levels) (Table S1, Supporting information). However, when

* Corresponding author at: Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, MD 20742, United States.
E-mail address: gpayne@umd.edu (G.F. Payne).

http://dx.doi.org/10.1016/j.bios.2017.04.008
Received 5 January 2017; Received in revised form 29 March 2017; Accepted 10 April 2017
Available online 12 April 2017
0956-5663/

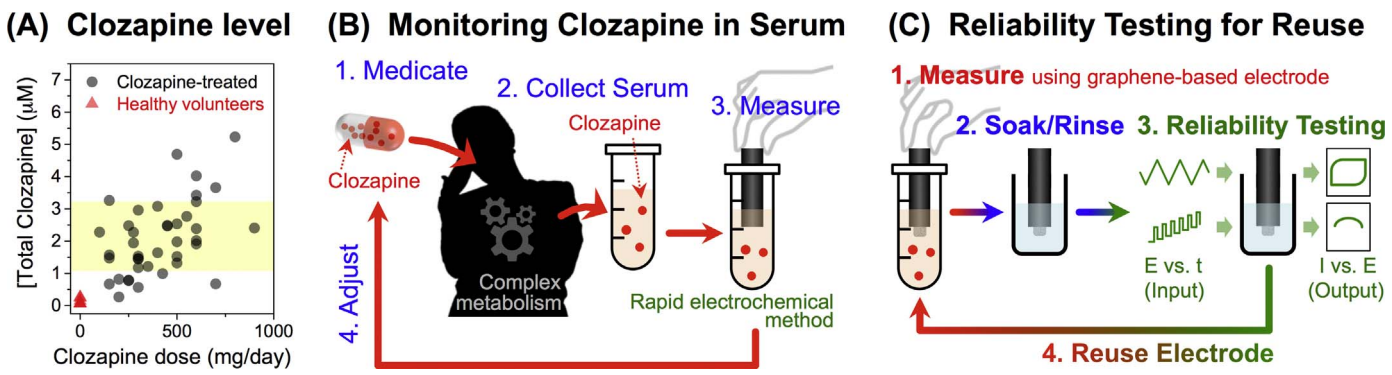

Fig. 1. Rapid therapeutic drug monitoring for the antipsychotic medication clozapine. (A) Clozapine is challenging to prescribe because it is active a low concentrations with narrow therapeutic window (1–3 μM), and it is difficult to control serum levels by dosage because of significant variabilities in its metabolism. Total clozapine was measured from centralized laboratory and is the sum of clozapine and norclozapine. (B) Electrode measurements offer the potential for simple and rapid measurement of clozapine in human serum. (C) Experimental procedure for reusing a graphene composite-coated electrode.

these methods are extended to clozapine-spiked serum analysis, sensitivities typically decrease by orders-of-magnitude (increases in limit of detection). To date, we are unaware of any method that has succeeded in the clinic to provide the timely information needed to adjust clozapine dosages and personalize the management of schizophrenia.

Here, we report serum clozapine analysis with an electrode coated with an electrodeposited graphene-chitosan composite coating. Because of our translational aim, we focus on clinical serum analyses (Chan et al., 2016) from three groups, healthy controls, and schizophrenia groups with and without clozapine-treatment. Traditional thinking would suggest that the problems in extending analysis from buffer to serum are that serum contains components that foul the electrode or interfere with the analyte's signal (Barfidokht and Gooding, 2014; Capaldo et al., 2016; Coldur and Andac, 2013; Downard and bin Mohamed, 1999; Geise et al., 1991). Our evidence indicates that neither is the problem. Rather, we believe the problem in extending analysis from buffer to serum is the large and possibly variable binding of clozapine to serum proteins (90–95% of clozapine is bound to serum proteins (Espnes et al., 2012; Flanagan et al., 2003; Leung et al., 2014; Schaber et al., 1998; Wu et al., 2011)). Presumably, the unbound clozapine is measured electrochemically which may explain the diminished sensitivities when buffered solutions are compared against clozapine-spiked serum samples (Kim et al., 2015). It has also been suggested that only the unbound form of clozapine is biologically active (Lee et al., 2016), yet standard centralized laboratory analyses measure the total clozapine (protein bound plus unbound). While we acknowledge the limitations of this standard analytical method, these measurements are currently used by clinicians to adjust dosages and thus we use this standard to develop our electrochemical method.

The goal of this work is to develop a simple, rapid and reliable method for serum clozapine measurement within the constraint that we are comparing against a standard method that is not entirely comparable (standard method measures bound and unbound forms of clozapine). We demonstrate the validity of our electrochemical measurements using uric acid as a proxy, and show a high correlation between electrochemical measurements and standard centralized laboratory analysis of uric acid ($r=0.960$, $p < 0.001$). As illustrated in Fig. 1**c**, we devised simple, automatable electrochemical reliability tests to ensure reliable measurements over extended electrode use. We specifically report the following advantages for this electrochemical serum clozapine measurement: (i) serum pre-treatment and dilution are not required; (ii) selectivity and sensitivity (limit of detection 0.7 μM) are appropriate; (iii) measurements are rapid (20 min); (iv) the graphene-chitosan coating is stable allowing repeated use over several weeks; and (v) rapid, automatable electrochemical reliability tests can be used to detect common error-causing problems.

2. Materials and methods

2.1. Chemicals

Chitosan, clozapine, norclozapine, and uric acid were purchased from Sigma-Aldrich. Graphene (N002-PDR) was purchased from Angstron Materials. Deionized water (> 18 MΩ) was obtained from Super-Q water system (Millipore). Chitosan solution (1.1%; pH 5.3) was prepared by dissolving chitosan flakes in deionized water and the pH was adjusted using an HCl solution. A stock solution of clozapine (5 mM) was prepared in methanol and stored at −80 °C. Standard clozapine solutions were prepared by diluting this stock solution with serum of healthy volunteers.

2.2. Fabrication of graphene-chitosan-coated electrode

Graphene-chitosan composite is deposited on a gold electrode, relying on the pH-responsive film-formation of chitosan (see Supporting Information for further details of the electrodeposition of the graphene-chitosan film) (Wan et al., 2011; Yang et al., 2013).

2.3. Instrumentation

To electrodeposit graphene-chitosan film, a DC power supply (2400 Sourcemeter, Keithley) was used. To perform electrochemical analyses, an electrochemical analyzer (CHI420a, CH Instruments) was used with a three-electrode configuration (Ag/AgCl (3 M KCl) reference electrode; Pt wire counter electrode). Differential pulse voltammetry was performed by scanning potential from 0 to 0.45 V at a scan rate of 2 mV/s (1 mV step increment; every 0.5 s) with superimposed pulses (50 mV pulse amplitude; 0.2 s pulse period). Cyclic voltammetry was performed by scanning potential from 0 to 0.6 V at a scan rate of 50 mV/s.

2.4. Clozapine analysis in serum

Blood samples from 3 populations were collected at the Maryland Psychiatric Research Center, University of Maryland School of Medicine, between May 2015 and August 2016 and serum samples were collected from the supernatant of the centrifuged blood of each participant and stored frozen (−80 °C) before assay. Those sampled populations include 1) individuals with a DSM-IV diagnosis of Schizophrenia or Schizoaffective Disorder, who are taking clozapine, 2) individuals with either diagnosis, but not taking clozapine and 3) a control group consisting of individuals with neither diagnosis nor taking clozapine. All participants completed data collection procedures on a single appointment lasting 1–2 h, and involved up to 45 mL of blood collection for commercial and electrochemical detection of