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Voltammetric monitoring of linezolid, meropenem and theophylline in plasma

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ABSTRACT

Treatment of healthcare associated Pneumonia (HCAP) caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) requires therapeutic protocols formed of linezolid (LIN) either alone or in combination with meropenem (MERO) and theophylline (THEO). The inter-individual pharmacokinetic variations require the development of reliable therapeutic drug monitoring (TDM) tools especially in immunocompromised patients. A sensitive square wave voltammetric sensor using multiwalled carbon nanotubes (MWCNTs) modified carbon paste electrode in Britton-Robinson buffer was developed and validated. Experimental parameters such as pH, percentage of MWCNTs, and pre-concentration time were optimized. The sensor was employed at pH 11.0 for the determination of LIN in plasma within a concentration range of 2.5×10^{-8} - 8.0×10^{-6} mol L^{-1}without interference from co-administered medications. On the other hand, simultaneous monitoring of LIN, MERO and THEO in plasma was feasible at pH 3.0 over concentration ranges of 4.0×10^{-7}- 9.0×10^{-5}, 8.0×10^{-7}- 9.0×10^{-5} and 8.0×10^{-7} - 9.0×10^{-5} mol L^{-1}, respectively. The performance of the proposed sensor was validated and the applicability for TDM has been demonstrated in plasma of healthy volunteers.

Introduction

Nosocomial infections caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) are significant causes of morbidity and mortality in hospitalized patients worldwide. MRSA has been associated with several hospital outbreaks since late 1970s and up to 60% of nosocomial infections in ICU [1]. MRSA is endemic to the Mediterranean region [2] and Egypt scored the highest MRSA clinical isolates as reported by several studies [3]. Vancomycin has been the first line of treatment for MRSA infections, but the emergence of Vancomycin-resistant strains of staphylococcus aureus [4], in addition to its nephrotoxicity limited its use. These drawbacks attracted the attention towards a novel antibiotic; linezolid (LIN) that has been approved by the FDA for treatment of skin and soft tissue infections caused by MRSA, community-acquired or healthcare associated pneumonia (HCAP) and vancomycin-resistant Enterococcus faecium infections [5].

HCAP accounts for 20–40% of all diagnosed cases of both hospital-acquired pneumonia and ventilator-associated pneumonia [6]. The current guidelines issued jointly by the American Thoracic Society and the Infectious Diseases Society of America recommended LIN as the first line of treatment [7]. However, the contribution of several microorganisms in pneumonia makes it difficult to depend on one antibiotic only. LIN-sensitive MRSA and meropenem (MERO)-sensitive *Klebsiella pneumonia* are the most commonly isolated bacteria. Thus, an empirical therapy of the two antibiotics would be fruitful. Addition of theophylline (THEO) in pneumonia treatment protocols is a common practice for its bronchodilator, and anti-inflammatory effects [8].

Dose adjustment especially in immunocompromised HCAP patients is extremely difficult due to inter-individual variations, narrow therapeutic window, and the presence of multiple factors that influence pharmacokinetics and pharmacodynamics of the prescribed drugs [9]. Therapeutic drug monitoring (TDM) is crucial for both LIN and MERO to maintain therapeutic levels above the minimum inhibitory concentration (MIC) to ensure efficacy and avoid the evolution of resistant bacterial strains. THEO is also known for its narrow therapeutic window [10] and death due to cardiotoxicity, neurotoxicity, or seizures have been reported with THEO levels above 15 mg L^{-1} [11].

In literature, several chromatographic methods using HPLC-UV and tandem mass spectrometry have been reported for the analysis of LIN, MERO and THEO individually in biological fluids. For LIN, HPLC-UV methods [12–14], UPLC-PDA [15], and LC-MS/MS [16–18] have been reported. Concerning MERO, LC-MS/MS [19], HPLC-UV [20], UHPLC-PDA [21] were applied. While LC-MS/MS [22], HPLC-voltammetry

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[23] and HPLC-UV [24] were used in the case of THEO. Spectrophotometric and spectrofluorimetric methods [25–29] were used to determine LIN.

Electrochemical techniques are emerging tools for TDM in clinical settings. Voltammetry in particular is simple and cost-effective technique that is capable to deliver accurate, sensitive and reliable results in short analysis time. Carbon paste electrode (CPE) is ecofriendly, available and cheap electrode characterized by the easily renewable surface, low background current, low resistance and minimum sample requirements. Various modifiers have been reported to enhance the functionality of CPE including carbon nanotubes (CNTs) that play important role in improving mechanical, chemical and physical properties of the CPE [30]. It has been reported also that CNTs can enhance the electrocatalytic activity of the electrode by promoting electron transfer reactions [31].

Voltammetry has been employed in several reports for the determination of LIN, MERO and THEO individually in biological fluids [32–38]. To the best of our knowledge, only one method has been reported for the simultaneous determination of LIN and MERO using UHPLC/PDA [39] and applicability to THEO determination in the same samples has not been investigated.

The determination of drugs at higher sensitivity than the reported methods is needed, therefore in the present study; a rapid, economical, simple, precise and sensitive voltammetric method is applied for TDM of LIN in plasma at pH 11.0; with no interference from the co-administered drugs or at pH 3.0 for simultaneous determination of LIN, MERO, and THEO in plasma of healthy volunteers using a MWCNTs modified CPE (MWCNTCPE). The developed sensor was highly sensitive, reliable and can be further employed to study the pharmacokinetics of the studied drugs using square wave voltammetry (SWV). The ease of performance of the test allows routine and continuous monitoring for all patients and better clinical outcomes. The proposed method can be used for routine quality control of the drugs in resource-limited countries.

Experimental

Materials and reagents

All regents and solvents were of analytical reagent grade and were used without further purification.

Pure materials

Linezolid ({(S)-N-({3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl} methyl)acetamide} [40], meropenem trihydrate {(4R,5S,6S)-3-[(3S,5S)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]sulfanyl-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid} [40], and theophylline {1,3-dimethyl-7H-purine-2,6-dione} [40] were supplied by Averroes pharma, AstraZeneca, and El-Nile pharmaceuticals Co, respectively. The purity was checked according to the USP 2016 and was found to be 99.60%, 99.92%, and 99.71%, respectively.

Market samples

Averzolid tablets (600 mg of LIN/tablet, Batch No.151091) produced by El-Obour Modern Pharmaceutical Co., Meropenem vials (1 g of MERO/vial, Batch No.LW544) by AstraZeneca UK Limited, and Uniphylline capsules (400 mg of THEO/capsule, Batch No.653907) by El-Nile pharmaceuticals Co. were purchased from local market.

Reagents

Britton-Robinson (BR) buffer solutions were used as the supporting electrolyte. BR buffers of different pH (2.0–11.0) were prepared as described in literature [41].

Graphite powder, paraffin oil, methanol, ether, and acetonitrile were supplied from (Sigma-Aldrich, USA). Multiwalled carbon nanotubes (MWCNTs) of purity > 95.0% were purchased from NanoLab (USA). Human blank plasma was obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Egypt). Real samples were obtained from healthy volunteer.

Standard solutions

The standard stock solutions of LIN, MERO, and THEO (1.0×10^{-2} mol L^{-1}) were prepared by dissolving accurately weighed amounts in methanol for LIN and in double distilled water for MERO and THEO. The stock solutions were stored in amber-colored bottles and kept refrigerated for up to 7 days.

Apparatus

All voltammetric measurements were carried out at room temperature using a PC-controlled analytical electrochemical workstation (SP-150, Biologic Science Instruments, France) with electrochemistry software (EC lab). The reference electrode Ag/AgCl/3 mol L^{-1} NaCl (MW-2063, BASi model, USA), a platinum wire counter electrode (MW-1032, BASi model, USA) and glass cell (5.0 mL) were used for electrochemical measurements. A Jenway 3510 pH meter (Staffordshire, England) with glass combination electrode was used for pH measurements.

Procedures

Preparation of bare carbon paste electrode (CPE)

Graphite powder (500 mg) was levigated with paraffin oil (~0.3 mL) in a glass mortar till a homogenous paste is obtained. The paste was then firmly packed into the hole of the electrode approximately 3.0 mm and smoothed on a filter paper until a shiny appearance was obtained.

Preparation of modified electrode

The modified electrode was then prepared by adding 3.0% MWCNTs (15 mg) to graphite powder (485 mg) in ether till homogeneity was obtained. The mixture was sonicated and the ether was allowed to evaporate, and then paraffin oil (nearly 0.3 mL) was added to obtain the paste.

The area of working electrode (MWCNTCPE) was obtained utilizing the cyclic voltammetry of 1.0×10^{-3} mol L^{-1} $K_3Fe(CN)_6$ in 0.1 mol L^{-1} KCl as supporting electrolyte at different scan rates through Randles-Sevcik equation: $Ip = (2.69 \times 10^5)\ n^{3/2}\ A\ C_o^*\ D_o^{1/2}\ \upsilon^{1/2}$, where Ip is the anodic peak current (A), D_o is the diffusion coefficient (7.6×10^{-6} cm^2 s^{-1}), υ is the scan rate (V s^{-1}), n is the number of electrons exchanged during electrode reaction (n = 1), and C_o^* is the concentration of $K_3Fe(CN)_6$. Using slope of the plot of Ip versus $\upsilon^{1/2}$ A was calculated to be 0.896 cm^2. The surface area of CPE was calculated using the same procedures to be 0.095 cm^2 [42].

Scanning electron microscope (SEM) for both electrodes was performed using JSM-6700F scanning electron microscope (Japan Electro Company, Japan).

Application to therapeutic drug monitoring

Determination of LIN in spiked plasma. Plasma samples were stored frozen at −86 °C and all analytical procedures were carried out at room temperature. Aliquots of 1.0 mL plasma were placed in 10.0 mL centrifuge tube and fortified with different volumes of standard LIN solution (5.0×10^{-4} mol L^{-1}) as described in more detail below. Plasma protein precipitation was carried out by adding 3.0 mL acetonitrile and the mixture was then centrifuged for 15.0 min at 5000 rpm then the supernatant was carefully transferred into 5.0 mL