Talanta 113 (2013) 36–40

Contents lists available at SciVerse ScienceDirect

Talanta

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

An electrochemical biosensor for clenbuterol detection and pharmacokinetics investigation

Bing Bo [a,b], Xuejun Zhu [b], Peng Miao [b], Dong Pei [a], Bo Jiang [c], Yue Lou [c], Yongqian Shu [a,*], Genxi Li [b,d,**]

[a] Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, PR China
[b] Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, PR China
[c] Department of Pediatric Orthopaedics, Nanjing Children's Hospital Affiliated to Nanjing Medical University, Nanjing 210008, PR China
[d] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, PR China

ARTICLE INFO

Article history:
Received 13 December 2012
Received in revised form
15 March 2013
Accepted 23 March 2013
Available online 30 March 2013

Keywords:
Biosensors
Clenbuterol
Electrochemical techniques
Nanoparticles
Pharmacokinetics

ABSTRACT

Clenbuterol is a member of β2 adrenergic agonists, which is widely used not only as a food additive for livestocks, but also a kind of stimulant for athletes; however, the abuse of clenbuterol may pose a significant negative impact on human health. Since it is highly required to develop fast, sensitive and cost-effective method to determine clenbuterol level in the suspected urine or blood, we herein have fabricated an electrochemical biosensor for the determination of clenbuterol. Measurement of the species with the proposed biosensor can also have the advantages of simplicity, high sensitivity and selectivity. Moreover, the sensor can be directly used for clenbuterol determination in rat urine. We have further studied the pharmacokinetics of clenbuterol by using this proposed electrochemical biosensor, so a new tool to investigate pharmacokinetic is developed in this work.

1. Introduction

Clenbuterol is a member of β2 adrenergic agonists, which has been known as a thermogenic drug; however, this kind of β2 adrenergic has also been popularly abused as a food additive for livestocks and stimulant for athletes, due to its omnipotent capacity to improve growth rate and reduce carcass fat [1,2]. Nevertheless, even a tiny exposition to the β adrenergic agonist either from athletic stimulants or from animal product (mainly from pork), may pose severe threat to humans, causing acute poisoning with symptoms of muscular tremor, cardiac palpitation, vomiting, nausea, nervousness and chills [3]. Due to the abominable side effects of clenbuterol, this drug is banned in most countries. To eliminate the abuse of clenbuterol, it is of high necessity and great significance to develop fast, sensitive, and cost-effective methods to determine clenbuterol levels in the suspected urine or animal product. In the meantime, better understanding of the pharmacokinetics of clenbuterol is needed, since it may help guide clinical medication safety, prevent adverse reactions, which may further contributes to unequivocal diagnosis of clenbuterol food poisoning.

So far, many methods by using different detection techniques have been proposed for clenbuterol determination, such as liquid chromatography, mass spectrometry (MS), gas chromatography coupled with MS, capillary electrophoresis with amperometric detection, colorimetric assay, immunoassay and surface-enhanced Raman scattering (SERS) [4–11]. However, the complicated pre-purification procedures, high cost and long testing period have limited their wide applications [12]. Therefore, more easily and conveniently operated, cost-and-time effective methods are still highly required to be developed.

Biosensors, especially electrochemical biosensors, with superb advantages of time-effectiveness, simple procedures and high sensitivity, have received significant scientific and industrial interests [13–15]. Especially in recent years, due to the achievements of surface assembly and modification, as well as the use of nanomaterials, more and more functionalized electrochemical biosensors are fabricated. In this work, based on the formation of platinum nanoparticles (PtNPs), which can perform high electrocatalytic activity toward the reduction of H_2O_2 to simplify analytical procedures [16], we have fabricated an electrochemical biosensor

Abbreviations: MS, Mass spectrometry; SERS, Surface-enhanced Raman scattering; PtNPs, Platinum nanoparticles; CTAC, Cetyltrimethylammonium chloride; 1,4-BDT, 1,4-Benzenedithiol; SD Rats, Sprague-Dawley rats; SCE, Saturated calomel reference electrode; DPV, Differential pulse voltammetry; EIS, Electrochemical impedance spectroscopy; CA, Chronoamperometry; SAM, Self-assembled monolayer.

* Corresponding author. Fax: +86 25 83710040.
** Corresponding author at: Nanjing University, Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, No. 22 Hankou Road, Nanjing 210093, PR China. Tel.: +86 25 83593596; fax: +86 25 83592510.
E-mail addresses: shuyongqian@csco.org.cn (Y. Shu), genxili@nju.edu.cn (G. Li).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.03.056

for the determination of clenbuterol. The fabricated biosensor cannot only be used for clenbuterol determination in rat urine, but also enables us to understand the pharmacokinetics of clenbuterol in rat urine without any signal interruptions [17]. So, the fabricated biosensor for clenbuterol determination proposed in this work may also hold considerable potentials to the development of next-generation strategy for pharmacokinetic study, in addition to the unequivocal diagnosis of food poisoning in the future.

2. Experimental

2.1. Materials and chemicals

Clenbuterol, cetyltrimethylammonium chloride (CTAC, 25% in water), chloramphenicol, ampicillin, kanamycin and glucose were obtained from Sigma-Aldrich. 1,4-Benzenedithiol (1,4-BDT) was purchased from Alfa-Aesar. H_2O_2, $H_2PtCl_6 \cdot 6H_2O$, $Na_2B_4O_7 \cdot 10H_2O$ were ordered from Nanjing Chemical Co., Ltd. Other reagents were of analytical reagent grade and used as received. Unless otherwise noted, all solutions were prepared with double-distilled water that was purified with a Milli-Q purification system (Barnstead, USA) to a specific resistance of >18 MΩ cm. Female Sprague-Dawley rats (SD) of about 6 weeks age were provided by Nanjing Medical University (Nanjing, China).

The buffer solutions employed in this work were as follows: 1,4-BDT buffer (1 mM 1,4-BDT diluted in ethanol), reaction buffer (4.8×10^{-7} M H_2PtCl_6, 2 mM CTAC, 0.01 M $Na_2B_4O_7$, pH 10.0), working buffer (0.1 M PBS, 10 mM H_2O_2, pH 7.4).

2.2. Preparation of rat urine samples

All animal protocols were performed in accordance with institutional and national guidelines and with the approval by the Animal Care Ethics Committee of Nanjing Medical University. Female SD Rats of about 6 weeks age were provided by Experimental Animal Centre of Nanjing Medical University (Nanjing, China). The rats were raised in metabolic cages for 2 weeks in a controlled environment (23 ± 3 °C with the humidity of 40–60%, 12 h light–dark cycle with lighting from 8:00 am to 8:00 pm). Rats were allowed to have free access to rat chow and water. All rats were randomly grouped into two groups (10 rats per group): an experimental group and a control group. A single dose of 5.0 mg/kg clenbuterol (1 mg/mL in double-distilled water) was intragastricly administrated to each of the female rats in the experimental group. The rats were fasted for more than 12 h before drug administration. The rats used as control groups were administrated with double-distilled water instead after 12 h fastness. The urine samples were collected in aseptic tubes by bladder area stimulation. The collection procedure was performed at 2, 5, 6, 8, 9, 12, 24 h after drug administration. The samples were stored in −20 °C before the electrochemical measurements. The whole process was conducted in aseptic environment, and all the materials and apparatuses were germ free [18,19].

2.3. Preparation of 1,4-BDT modified gold electrode

First, the gold electrode (3 mm diameter) was polished carefully on P3000, P5000 sand paper and alumina slurry (1 μm, 0.3 μm, 0.05 μm), respectively. Subsequently, it was thoroughly washed by ultra-sonicating in both ethanol and double-distilled water to remove the residuals, each for about 5 min. Piranha solution (98% H_2SO_4:30% H_2O_2=3:1) was then carefully dropped on the gold electrode surface (*Caution*: *Piranha solution reacts violently with organic solvents and should be handled with great care*!). After that, the electrode was further incubated in HNO_3 for 30 min, followed by thoroughly ultra-sonicating in both ethanol and double-distilled water for about 5 min. Finally, it was electrochemically cleaned with 0.5 M H_2SO_4 to remove any remaining impurities. After being dried with nitrogen, the electrode was immediately used for 1,4-BDT immobilization for 16 h at room temperature.

2.4. Fabrication of PtNPs assembled electrode surface

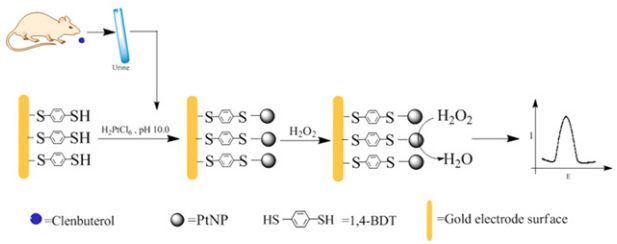
The pre-modified gold electrode was first washed by 5 mL ethanol and water separately in order to remove any non-covalently attached 1,4-BDT. Then it was immersed in the reaction buffer containing target clenbuterol or rat urine for 90 min at 70 °C. PtNPs could be synthesized on the electrode surface in the presence of clenbuterol. After that, the gold electrode was thoroughly washed by double-distilled water.

2.5. Electrochemical measurements

Electrochemical measurements were carried out on an Electrochemical Analyzer (CHI660C, CH Instruments). A three-electrode system was employed, which consisted of the modified gold electrode as the working electrode, a saturated calomel reference electrode (SCE) and a platinum auxiliary electrode. Differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS) and chronoamperometry (CA) were all performed at room temperature. The scan range of DPV was from −0.5 V to 0.1 V, and the initial potential was set to be −0.3 V for CA. For EIS, 5 mM $Fe(CN)_6^{3-/4-}$ with 1 M KCl was employed as the electrolyte. The spectra were recorded by applying a bias potential of 0.259 V vs. SCE and 5 mV amplitude in the frequency range of 0.1–100 kHz. Each measurement was repeated for at least three times.

3. Results and discussion

Scheme 1 may illustrate the principle of the electrochemical biosensor proposed in this work for clenbuterol determination and pharmacokinetics investigation, while urine samples are collected in aseptic tubes every a few hours after drug administration. First, 1,4-benzenedithiol (1,4-BDT), an adapter molecule, is covalently immobilized on the gold electrode surface, contributing to an arranged and compact self-assembled monolayer (SAM) [20], which also leads to a remarkable electron transfer resistance. Subsequently, the reaction between chloroplatinic acid and clenbuterol occurs, involving the oxidation of the amine groups of clenbuterol and the reduction of chloroplatinic acid into atomic platinum [21]. PtNPs are then formed, which has been confirmed by transmission electron microscope (Fig. S1). The formed PtNPs can then make a decrease of the electron transfer resistance (Fig. S2) [22]. Moreover, since PtNPs can electrochemically catalyze the reduction of H_2O_2, notable electrochemical signals can be generated to indicate the existence of clenbuterol in the test



Scheme 1. Schematic illustration of the biosensor for clenbuterol determination and pharmacokinetic investigation in rat urine.