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Voltammetric and spectroscopic studies on the binding of the antitumor drug dacarbazine with DNA

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ABSTRACT

The interaction of dacarbazine (DTIC) with salmon sperm double-stranded DNA (ss-ds-DNA) was studied in phosphate buffer (PB) solution of physiological pH 7.4 by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at screen-printed carbon electrode (SPCE). UV–vis absorption spectroscopic technique was also employed to probe the interaction between the DTIC and ss-ds-DNA in solution. The binding of DTIC with DNA, immobilized onto the surface of an anodically activated SPCE, was exploited for designing a sensitive biosensor for DTIC. A detection scheme based on a preconcentration and DPV determination at ss-ds-DNA modified SPCE (DNA/SPCE) was proposed for the trace determination of the DTIC in spiked human serum samples.

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1. Introduction

Dacarbazine (DTIC, 5-(3,3-dimethyl-l-triazeno)-imidazole-4-carboxamide, Scheme 1) has for a long time been used as an antitumor agent. The single-agent DTIC remains the standard chemotherapy [1]. No multiagent chemotherapy has yet proved superior to single-agent DTIC chemotherapy in phase III clinical trials [1–3]. DTIC has also been used in combination with other drugs for treating renal adenocarcinoma, soft tissue sarcoma, malignant lymphomas, brain cancer and Hodgkin's disease [4]. DTIC is an active prodrug for the treatment of advanced malignant melanoma [5], Hodgkin's lymphoma [6,7], soft-tissue sarcomas [6,8,9], and childhood solid tumors [8,10]. DTIC is the precursor of 5-(3-methyl-1-triazenyl) imidazole-4-carboxamide (MITC) and its byproduct the methyldiazonium ion is responsible for alkylation. DTIC is activated in the liver to yield the highly reactive methyl diazonium cation. The formation of this cation is considered as the major mechanism for its antitumor effect. Methyl diazonium intermediate alkylates the N-7 of guanine in DNA [11]. DNA methylation by DTIC [12,13] occurs on adenine N3 and guanine N7 and O6, the latter being responsible for the major part of its cytotoxicity. O6-methyl guanine is mutagenic due to its mispairing with thymine and subsequent G:C to A:T transitions, the persistence of which leads to cytotoxic effects by the generation of secondary strand breaks [12,13].

Stability and compatibility assays of pharmaceutical formulations of DTIC by LC-UV [14–17] and LC-MS [18] have been described. LC-UV [19,20] and LC-MS/MS [21] methods have also been used for the quantification of DTIC and its degradation products in urine and plasma. Due to the extreme hydrophilic and unstable character of DTIC and its terminal metabolite (5-amino-4-imidazole-carboxamide), HILIC-MS/MS method with a two-step extraction process was considered as specially adapted for the analysis of these compounds in human plasma [21]. The method was validated and presented good quantitative performance in terms of accuracy, precision and specificity with an LOQ of 0.5 ng mL^{-1} allowing PK studies. With LC-UV method preceded by simple protein precipitation (methanol), PK studies were also possible, however, LOQ in plasma samples of DTIC and its metabolites were superior (about 30 ng mL^{-1} for DTIC) with a RSD of 20% [20].

The study of small drug molecules interacting with nucleic acids is an area of intense research that has particular relevance in our understanding of relative mechanism in chemotherapeutic applications and the association between genetics (including sequence variation) and drug response. Different techniques have been used to investigate the binding interactions of drugs with DNA, including: UV–vis spectrophotometry [22]; fluorescence spectroscopy [23], constant wavelength synchronous fluorescence spectroscopy (CW-SFS) [24], circular dichroism [25], resonance Raman spectroscopy [26], Fourier transform infrared spectroscopy [27] and single-molecule force spectroscopy [28]. The mechanism of interaction between drugs and DNA can be explained electrochemically by two different ways; DNA-modified electrode and interaction in solution [29–32]. The electrochemical techniques are characterized by high sensitivity, low cost, simplicity, easy handling, rapidness, compatibility with microfabrication technology

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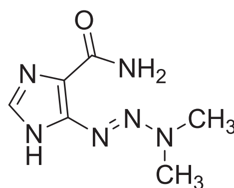

Scheme 1.

and require small amount of sample, thus offering advantages over commonly used biological and chemical assays. The electrochemistry of DTIC has also been studied using d.c. polarographic, Tast and differential-pulse polarography, adsorptive stripping voltammetry cyclic and differential pulse voltammetry and with h.p.1.c. with oxidative amperometry at a glassy carbon electrode and their application to the trace determination of DTIC [33]. The electrochemical behavior of the antitumor agent DTIC and its major metabolite, 5-aminoimidazole-4-carboxamide (AIC), on carbon paste electrodes has been studied [34]. The electrochemical properties and the DNA/DNA base binding behavior of DTIC drug, has been investigated in previous reports [35,36].

In this article, the interaction of DTIC with ss dsDNA has been studied by CV and DPV in solutions of physiological pH 7.4 at SPCEs. The diffusion coefficients for both the free and bound DTIC (D_f, D_b), the binding constant (K) of DTIC with DNA were determined. The nature of the interaction was explored. UV–vis absorption spectroscopy was also used to delineate the interaction mechanism of DTIC with DNA. The advantages of SPCEs include low cost, potential for miniaturization, facility of automation, and easy construction of simple and portable equipment. Furthermore, interaction of DTIC with ss dsDNA immobilized at SPCE surface has also been investigated. An analytical procedure for the determination of DTIC in serum at the dsDNA–SPCEs biosensor has been developed.

2. Experimental

2.1. Apparatus

Voltammetric measurements were carried out using CHI610C Electrochemical Analyzer controlled by CHI Version 9.09 software (CH Instruments, USA). A three-electrode configuration was composed of a working screen-printed carbon electrode (3.1 mm diameter), printed from a carbon-based ink; a silver–silver chloride pseudo-reference electrode made from a silver-based ink; and the auxiliary electrode from a carbon ink. All pH-metric measurements were made on a CG 808 (Schott Gerate, Germany) digital pH-meter with glass combination electrode, which was previously standardized with buffers of known pHs. The UV spectra were performed by a PerkinElmer UV–vis double beam spectrophotometer equipped with a PC for data processing UV WinLab-ver 2.80.03 (PerkinElmer, USA). The spectra were recorded over the wavelength range from 200 to 650 nm at a scan speed of 240 nm/min. A quartz cell with a 1.0 cm path length was used.

2.2. Chemicals and reagents

Salmon sperm DNA sodium salt (Sigma, D-1626, USA), containing 41.2% G–C base pairs with molecular weight $\approx 1.3 \times 10^6$ Da ($\approx$2000 base pairs) was used [37]. The DNA concentration (in moles of base pairs per liter) was determined from the intensity of the absorption at $\lambda = 260$ nm, using the molar absorption coefficient $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$ [38]. DTIC was obtained from Sigma and used as received without further purification. Stock solutions of DTIC (1×10^{-3} M) were prepared by dissolving an appropriate amount of the drug in de-ionized water and storing the solution in the dark at 4 °C. DTIC preparations are currently administered by intravenous injection or infusion. However, DTIC is unstable in aqueous solution. Previous study has demonstrated that this drug rapidly photodecomposes in sunlight. Electrochemical cells and volumetric flasks were protected from light to avoid DTIC photodecomposition [18,39]. All other chemicals used were analytical reagent grade. Triply distilled water was used in all solutions. Unless otherwise stated, all experiments were done in PB solutions (0.2 M, pH 7.4) at room temperature (25 °C).

2.3. Procedures

The CV, DP voltammograms or UV-absorption spectra of DTIC (fixed concentration) was recorded in presence of increasing concentrations of ss ds DNA in PB solutions (0.2 M, pH 7.4). After each addition of DNA to DTIC, the interaction time of 10 min was maintained and a CV, DPV or UV-absorption spectrum was recorded. The SPCE surface was pre-treated by applying +1.70 V for 1 min in PB solution (0.2 M, pH 7.4) without stirring, creating a surface on which DNA adsorbed through electrostatic interaction with carboxyl moieties [40]. The dsDNA was adsorbed on the surface of the pretreated SPCE by applying a potential of +0.30 V for 3 min in 33.0 $\mu g\ mL^{-1}$ DNA solution. Following washing with distilled water, DTIC was accumulated for selected times at open circuit at the dsDNA–SPCE by spreading of 50 µL of DTIC solution of different concentrations of DTIC (1.8, 3.6, 9.0, 18.0, and 90.0 ng/mL). The electrodes were rinsed with distilled water after DTIC incubation, (in 1.0 mL of 0.2 M PB, pH 7.4) onto the electrode surface and a 50 µL of DTIC free PB solution was dropped onto the surface of the sensor and the DPV was recorded with the following instrumental settings: pulse amplitude 50 mV; pulse width 30 s and sample width 0.02 s. For the determination of DTIC in serum: 1.0 mL of spiked serum was transferred to an eppendorf tube. After mixing, the precipitate was separated by centrifugation for 5 min. Then, 100 µL of the solution phase were transferred to another tube and completed to a final volume of 1.0 mL with 0.2 M PB pH 7.4, and 50 µL of the resulting solution were pipetted onto the DNA–SPCE. After the accumulation, the electrode was rinsed and covered with 50 µL drug-free 0.2 M PBpH 7.4 and then the DPV was recorded. The standard additions method was used for the quantitation. For ionic strength studies, interaction of DNA with DTIC were carried out in 20 mM buffer (pH 7.4) and increasing KCl concentrations in the range of 0.0 mM (low-salt) to 150.0 mM (high-salt).

3. Results and discussions

3.1. Investigation of interaction of DTIC with DNA in solution

The typical cyclic voltammograms of 100.0 µM DTIC without and with dsDNA at SPCE in PB (0.2 M, pH 7.4) are shown in Fig. 1. In the forward scan, a single anodic peak is observed, which corresponds to the oxidation of DTIC, probably at the –NH group of the imidazole ring [34]. The compound is oxidized in a two-electron process at glassy carbon electrode to yield the final product, the 5-hydroxyimidazol-4-carboxamide. A likely mechanism for the oxidation of DTIC molecule at SPCE could be postulated. In the reverse scan, no cathodic peak is observed, which indicates that the process is irreversible. When salmon sperm dsDNA is added to a solution of DTIC, marked decreases in the peak current heights and shifts of peak potentials to more positive values are observed. To show that the decrease in peak current is due to diffusion of DTIC–DNA adduct and not due to blockage of the electrode surface

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