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Studies on the anticancer drug mitoxantrone–DNA–sodium dodecyl sulfate system

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ABSTRACT

The interaction of anticancer drug mitoxantrone with sodium dodecyl sulfate (SDS) in the presence of two fixed DNA concentrations (1.75×10^{-5} M, when electrostatic binding of positively charged mitoxantrone at negatively charged DNA prevails and 1.50×10^{-4} M, when mitoxantrone molecules intercalate between DNA base pairs) was investigated by UV–Visible absorption spectroscopy.

For both DNA concentrations, the spectral results indicate two processes: process I in premicellar SDS concentration assigned to electrostatic interaction between positively charged mitoxantrone and negatively charged SDS monomers, and process II in micellar SDS concentration when the surfactant micelles are formed and mitoxantrone is encapsulated in micelles as monomer. Absorption and circular dichroism spectroscopy, and thermal denaturation studies evidenced the exclusion of intercalated mitoxantrone from DNA and further their encapsulation in SDS micelles.

The values of the total binding free energies show that the interaction of $(\text{mitoxantrone–DNA})_{\text{complex}}$ with SDS is favored in the presence of low DNA concentration (−15.96 kcal/mol), when drug molecules are electrostatically bound to DNA, than in the presence of high DNA concentration (−10.62 kcal/mol) when drug molecules are intercalated between base pairs of DNA. This difference in the total binding free energies can be attributed to the cost of the deintercalation process of mitoxantrone from DNA.

1. Introduction

Mitoxantrone (1,4-dihydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]-anthracene-9,10-dione) is a synthetic anticancer analog of anthracycline antibiotics, with significant clinical effectiveness in the treatment of different human cancers [1–3] and multiple sclerosis [4,5]. Structurally mitoxantrone is symmetrical, containing a tricyclic planar chromophore substituted with two nitrogen-containing side chains, positively charged at physiological pH (Fig. 1).

Different studies on the mechanism of mitoxantrone action indicate that nuclear DNA is the major target and the planar anthraquinone ring intercalates between DNA base pairs and the nitrogen-containing side chains bind the negatively charged phosphate groups of DNA [6–12]. Also, mitoxantrone is a potent inhibitor of topoisomerase II, an enzyme known to be important for the repair of damaged DNA [13,14].

Previous studies suggest that mitoxantrone has less cardiotoxicity than anthracyclines, but further investigations reveal that cardiotoxicity can occur at any time during therapy, and the risk increases with increased cumulative dose [15,16]. Its clinical usefulness is also limited by the occurrence of multidrug resistance associated with the overexpression of membrane transporters [17]. Different drug delivery systems have been studied in an attempt to improve the antitumour effect of mitoxantrone and to prevent harmful side effects [18–21].

An important and fundamental event in the interaction of drugs with biological tissues at the molecular level is their binding to membranes, which is related to the mechanism of drug action. Because biological membranes are extremely complex structures, surfactant micelles with similar polar and hydrophobic regions in their structure but much less complexity have been used as simplified model of the membranes to investigate physico-chemical aspects of the drug binding [22–27]. As compared to other membrane models such as liposomes and soluble polymers, the micelles are considered to be more advantageous because of their simplicity, low toxicity, narrow size distribution, longer residence time in the system and the enhanced bioavailability and stability of drug through micelle incorporation [28–30]. Ionic (sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB)) and non-ionic surfactants (Triton X-100, Brij-35 and Tweens) are commonly accepted as model systems for studying different aspects of membrane interactions with drug molecules, including their localization [23,26,31]. As many biological processes occur at the ionizable surface of membranes or along their hydrophobic region, a comparative study of the drug interaction with cationic, zwitterionic, anionic and

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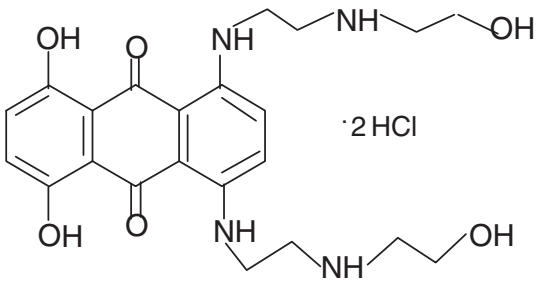

Fig. 1. Chemical structure of mitoxantrone.

neutral surfactants may provide useful information on the nature of the drug–membrane interaction [23–27,31].

In the context of the possibility of utilization of surfactant micelles as drug carrier [32] and to obtain information about the interaction with biological membranes, we performed a systematic investigation on the interaction of mitoxantrone with anionic [33], cationic [34] and non-ionic [35] micelles. Anionic and cationic micelles were chosen as a model of the lipid systems in order to study the contribution of different charges at the polar surfactant head groups (i.e. the electrostatic contribution), while the non-ionic micelles were chosen in order to clarify the hydrophobic contribution to the drug binding.

A detailed understanding of the mechanisms by which drug molecules recognize and bind to DNA and correlating them with biological effects is an important step in the rational drug design. The formation and dissociation process of drug–DNA complexes are of great therapeutic importance; for example for a drug to be efficient in cancer therapy one of the most important properties is to present an extremely slow rate of dissociation from DNA. The micelle-induced "deintercalation" phenomenon in which the presence of micelles draws out the intercalated drug molecules from the DNA interior and places them in the micelles was observed for different ligands [36–40]. This process may have significant importance in biological processes, taking into account that amphiphilic molecules are present in real biological systems: from the phospholipids in the cell membrane to polyamines such as spermine and spermidine in the nucleus [36]. A proper understanding of the deintercalation process would offer a substantial input towards specific therapeutic applications.

In this regard, the present study aims to investigate the interaction of mitoxantrone with DNA and anionic surfactant SDS in the presence of two fixed DNA concentrations: 1.75×10^{-5} M (when electrostatic binding of positively charged drug molecules at negatively charged phosphate groups of DNA prevails) and 1.50×10^{-4} M (when mitoxantrone molecules intercalate between base pairs of DNA) using UV–Visible absorption spectroscopy. The strategy adopted in the present work is to analyze the complex mitoxantrone–DNA–SDS system by reference to the corresponding pseudo-binary drug–DNA and drug–SDS systems investigated in the same experimental conditions, in order to outline the influence of different experimental parameters (ionic strength, surfactant and DNA concentration) and forces involved in the binding process.

2. Materials and methods

2.1. Materials

Mitoxantrone dihydrochloride, calf thymus DNA and sodium dodecyl sulfate were purchased from Sigma Aldrich and used without further purification. All experiments were performed at room temperature in phosphate buffer (pH 7.4, ionic strength 0.15 M) and deionized water (18.2 M Ω cm, Milli-Q water purification system) was used for the preparation of solutions. Mitoxantrone and DNA stock solutions prepared by dissolving an appropriate amount of compound in phosphate buffer were stored at 4 °C in the dark and use within 5 days. Before use, the stock solutions were diluted to desired concentrations and these concentrations were determined spectrophotometrically by using molar absorption coefficients: $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$ (per nucleotide) for calf thymus DNA and $\varepsilon_{660} = 19{,}500\ M^{-1}\ cm^{-1}$ for mitoxantrone [10]. The purity of DNA was verified by measuring the ratio of absorbances at 260 nm and 280 nm and it was found to be around 1.83, indicating that the DNA was free from protein contamination.

2.2. UV–Visible absorption spectroscopy

The absorption spectra were recorded by using a JASCO V-630 spectrophotometer equipped with the peltier-controlled ETCR-762 model accessory (JASCO Corporation, Tokyo, JP). The measurements were done using a matched pair of quartz cuvettes of 1.0 cm optical length. For DNA–mitoxantrone binding studies, absorption titration experiments were performed by keeping the concentration of drug constant and successive additions of concentrated DNA stock solution directly into a cuvette containing 2 ml mitoxantrone solution. The mixture was shaken well and the absorption spectra were registered after equilibration. In the case of mitoxantrone–DNA–SDS studies, a mitoxantrone solution containing a fixed DNA concentration (1.75×10^{-5} M and 1.50×10^{-4} M) was titrated with successive additions of SDS until no further change in absorbance spectra was observed. The spectral results are the average of 3 to 5 different experiments.

The absorbance values at 660 nm were corrected for dilutions and used to evaluate the binding constants. Binding constants were determined by nonlinear fitting of the spectral data assuming a 1:1 drug:DNA(SDS) interaction and 1:2 drug:SDS interaction using the following equations [41].

$$A = \frac{A_0 + A_b K[SDS]}{1 + K[SDS]} \quad (1)$$

$$A = \frac{A_0 + A_b K[SDS]^2}{1 + K[SDS]^2} \quad (2)$$

where A, is the measured absorbance of monomer (660 nm); A_0, the absorbance of the drug in the absence of DNA (SDS); and A_b, the absorbance of the drug bound to DNA (SDS).

In order to resolve the overlapping spectral components in the visible absorption spectra of mitoxantrone, the spectra were deconvoluted with Gaussian multi-peaks function in PeakFit 4.11 software (Systat Software Inc., Chicago, IL). The goodness of the fit was considered from the fitting parameter ($R^2 \sim 1$) and the symmetrical distribution of the residuals.

Linear and nonlinear fitting of the experimental data was performed using Origin 7.0 (MicroCal Software, Inc., Piscataway, NJ, USA) and Table Curve 2D v5.01 (Systat Software Inc., Chicago, IL) software.

2.3. DNA UV melting studies

Absorbance at 260 nm versus temperature curves (melting profiles) was measured by heating the samples (DNA, DNA in the presence of SDS, mitoxantrone and, mitoxantrone and SDS) at a rate of 1 °C/min over the range of temperature 10 and 105 °C. The melting temperature (T_m) is the midpoint of the hyperchromic transition as determined by the maxima of the first derivative plots.

2.4. Circular dichroism (CD) spectroscopy

CD experiments were performed using a Jasco J-815 CD spectropolarimeter. CD spectra of pure DNA, DNA in the presence of SDS, mitoxantrone and, mitoxantrone and SDS were recorded in the far-UV range (210–320 nm) using rectangular quartz cuvettes of 1.0 cm optical path length. The response time, scan speed, bandwidth and sensitivity