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Spectroscopic studies of the effects of anticancer drug mitoxantrone interaction with calf-thymus DNA

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ABSTRACT

Mitoxantrone (MTX) (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) is a synthetic antineoplastic drug, widely used as a potent chemotherapeutic agent in the treatment of various types of cancer. It is structurally similar to classical anthracyclines. Widespread interest in the anticancer agent mitoxantrone has arisen because of its apparent lower risk of cardio-toxic effects compared to the naturally occurring anthracyclines. In the present work, we investigated the interaction of mitoxantrone with DNA in the buffer solution at physiological pH using Fourier transform infrared (FTIR), UV–Visible absorption and circular dichroism spectroscopic techniques. FTIR analysis revealed the intercalation of mitoxantrone between the DNA base pairs along with its external binding with phosphate–sugar backbone. The binding constant calculated for mitoxantrone–DNA association was found to be $3.88 \times 10^5\ M^{-1}$ indicating high affinity of drug with DNA double helix. Circular dichroism spectroscopic results suggest that there are no major conformational changes in DNA upon interaction with drug except some perturbations in native B-DNA at local level. The present work shows the capability of spectroscopic analysis to characterize the nature of drug–biomolecule complex and the effects of such interaction on the structure of biomolecule.

1. Introduction

Mitoxantrone (Novantrone™) is a synthetic antineoplastic drug related to the anthracycline antibiotic group. It is widely used in the treatment of various types of cancer, specifically for metastatic breast cancer, acute myeloid leukemia, non-small cell lung cancer, non-Hodgkin's lymphoma and prostate cancer [1–6]. It was developed by a rational modification of an analog of ametantrone, in an attempt to find a compound that lacks the cardio toxic response, but retains the potent cytotoxicity of the anthracycline [5–8]. Structurally this drug is symmetrical, comprising of a tricyclic planar chromophore and two basic side chains (Scheme 1) [2,3,9,10]. Mechanism of action of mitoxantrone is not fully elucidated yet studies have shown that nuclear DNA is the prime target for the drug [3,6]. Drug accumulates in cells and concentrates in the nucleus, where it binds with DNA and causes DNA condensation, cross linking and strand breakage that leads to the inhibition of both DNA replication and RNA transcription [5]. In addition to this, mitoxantrone is a potent inhibitor of topoisomerase II, an enzyme known to be important for the regulation of superhelicity of DNA [2,6]. Despite many studies, it is not known which type of conformational changes occur in DNA upon mitoxantrone interaction. Biological importance of drug mitoxantrone allow to focus the attention on the importance of drug–DNA interaction studies. In the present work, we have investigated the mode and stability of mitoxantrone binding to DNA double helix using Fourier transform infrared (FTIR), UV–Visible and circular dichroism (CD) spectroscopic techniques.

Attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectroscopy is a powerful technique [11–15] and provides information about the conformational and structural changes in biological molecule and covalent and noncovalent modification due to interaction of ligand [16]. It is fast, yields a strong signal with a very small amount of sample [17,18]. Along with FTIR, circular dichroism (CD) spectroscopy is routinely used to study conformational changes of biomolecules [19].

In the present work, ATR–FTIR spectroscopy, FTIR difference spectroscopy, CD and UV–Visible absorption spectroscopy was used to delineate the interaction mechanism of mitoxantrone with DNA at physiological pH. The binding constant, mode of mitoxantrone binding with DNA and conformational transitions have been discussed along with the stability of drug–DNA complexes formed.

2. Materials and methods

2.1. Materials

Mitoxantrone and highly polymerized type I calf thymus DNA (sodium content 6%) were purchased from Sigma Aldrich

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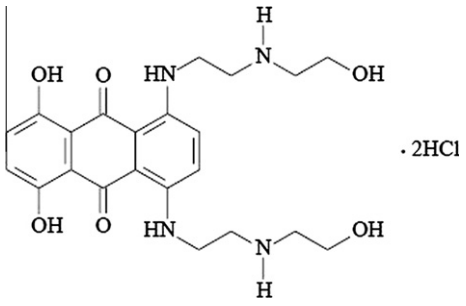

Scheme 1. Chemical structure of mitoxantrone.

chemicals (USA). Purity of DNA was determined by recording the UV absorbance at 260 nm (A_{260}) and 280 nm (A_{280}). The ratio of (A_{260})/(A_{280}) in DNA sample was found to be 1.82, which suggest that DNA is sufficiently free from protein impurities [20]. Other chemicals and reagents used in the study were of the analytical grade and used as supplied. For the preparation of buffer solution and mitoxantrone drug solution, deionized ultra pure water (resistance 18.2 MΩ) from Scholar-UV Nex UP 1000 system was used.

2.2. Preparation of stock solutions

Stock solution of DNA was prepared by dissolving 10 mg of calf thymus DNA per milliliter of Tris–HCl buffer (pH-7.4, 100 mM). The stock solution was kept at 8 °C for 24 h and stirred at the frequent intervals to ensure the homogeneity of DNA solution. Final concentration of the DNA stock solution was measured spectrophotometrically using extinction coefficient of 6600 cm^{-1} M^{-1} [21]. The concentration of DNA stock solution was adjusted to 24 mM (molarity of phosphate group). The mitoxantrone stock solution was prepared in ultra pure water and a series of dilutions of drug stock solution with varying concentration was prepared. Mitoxantrone solutions of different concentrations so prepared were added drop by drop to DNA solutions of constant concentration followed by continuous vortexing for 30 min. For FTIR measurements, solutions of three different concentrations were prepared containing 2.8, 0.8 and 0.48 mM of mitoxantrone with keeping constant DNA concentration (12 mM) that leads to the formation of mitoxantrone–DNA complex in the ratio of 1/15, 1/30 and 1/50 respectively. Circular dichroism studies were carried out using mitoxantrone concentration ranging from 0.1 to 0.3 mM and final DNA concentration of 2.5 mM. For the UV–Visible studies, DNA solution of 0.2 mM concentration was used with varying concentration of drug (1×10^{-1} to 3×10^{-2} mM).

2.3. FTIR measurements

FTIR spectra were recorded on a BIO-RAD FTS-175c spectrophotometer, equipped with deuterated triglycine sulfate detector and KBr beam splitter. Liquid sample were placed on ZnSe crystal tightly packed in micro-ATR assembly. Two hundred fifty-six scans were recorded for each sample in the spectral range of 2400–700 cm^{-1} with a resolution of 4 cm^{-1}. Spectra were baseline corrected and normalized for DNA band at 1086 cm^{-1}, the premier peak found in all the spectra [11,14–16]. Background spectra were collected before each measurement. A spectrum of buffer solution was recorded and subtracted from the spectra of DNA and mitoxantrone–DNA complexes. A satisfactory buffer subtraction was considered to be achieved when the intensity of water combination band at about 2200 cm^{-1} became zero in the spectra of DNA and mitoxantrone–DNA complexes [22].

2.4. CD spectral measurements

CD spectra of pure DNA and its complexes with mitoxantrone were recorded with JASCO J-815 CD spectrophotometer. All spectra were recorded in far-UV range (200–320 nm). Quartz cuvette with a path length of 1 nm was used for sampling. Three scans were recorded with a scan speed of 1 nm/s and averaged. Sample temperature was kept constant (25 °C) during entire experiment with the help of Peltier temperature controller. A spectrum of buffer solution was recorded and subtracted from the spectra of DNA and mitoxantrone–DNA complexes.

2.5. UV–Visible spectral measurements

The UV–Visible spectra of free DNA and its complexes with mitoxantrone were recorded on Perkin-Elmer Lambda 35 spectrometer. Quartz cuvette with a path length of 1 cm was used for the measurement. Binding constant for the association occurring between mitoxantrone and DNA was calculated using the method given by Kanakis et al. [23]. Calculated binding constant depends on the assumption that only one type of interaction occurs between DNA (D) and mitoxantrone (M) in aqueous solution resulting in the formation of one type of complex (DM) [24]. It is also presumed that the substrate and the ligand follow Beer's law for the absorbance of light. The absorbance of DNA solution (A_o) at its total concentration (D_t) with a path length (l) of 1 cm is

$$A_o = \varepsilon_D l D_t \quad (1)$$

where ε_D is the molar absorptivity of free DNA.

The absorbance of solution (A_M) comprising of total concentration of DNA (D_t) along with total concentration of mitoxantrone (M_t) is

$$A_M \rightarrow \varepsilon_D l[D] + \varepsilon_M l[M] + \varepsilon_{DM} l[DM] \quad (2)$$

where [D] is the concentration of uncomplexed DNA, [M] is the concentration of uncomplexed mitoxantrone, [DM] is the concentration of mitoxantrone–DNA complex, ε_M is the molar absorptivity of mitoxantrone and ε_{DM} is the molar absorptivity of mitoxantrone–DNA complex. After combining with the mass balance of DNA and mitoxantrone, the absorbance equation can be written as:

$$A_M \rightarrow \varepsilon_D l D_t + \varepsilon_M l M_t + \Delta\varepsilon_{DM} l[DM] \quad (3)$$

$$\Delta\varepsilon_{DM} \rightarrow \varepsilon_{DM} - \varepsilon_D - \varepsilon_M$$

The absorbance of solution (A) measured against the total concentration of mitoxantrone as reference is

$$A \rightarrow \varepsilon_D l D_t + \Delta\varepsilon_{DM} l[DM] \quad (4)$$

The stability constant (K_{DM}) for the formation of complex (DM) can be given as:

$$K_{DM} \rightarrow [DM]/[D][M] \quad (5)$$

Combining Eqs. (4) and (5)

$$\Delta A \rightarrow K_{DM}\Delta\varepsilon_{DM} l[D][M] \quad (6)$$

$$\Delta A = A - A_0$$

From the mass balance equation $D_t = [D] + [DM]$, we get $[D] = D_t/(1 + K_{DM}[M])$, which gives:

$$\frac{\Delta A}{l} \rightarrow \frac{D_t K_{DM}\Delta\varepsilon_{DM}[M]}{1 + K_{DM}[M]} \quad (7)$$

There is a hyperbolic relation between the free drug molecule concentration and its interaction with DNA. Linear transformation of Eq. (6) is done by taking the reciprocal of both side of Eq. (7) that gives: