International Journal of Biological Macromolecules 48 (2011) 87–92

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Interaction of mitoxantrone, as an anticancer drug, with chromatin proteins, core histones and H1, in solution

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ARTICLE INFO

Article history:
Received 22 August 2010
Received in revised form 2 October 2010
Accepted 4 October 2010
Available online 11 October 2010

Keywords:
Mitoxantrone
Core histone
Histone H1
Chromatin

ABSTRACT

In the present study, for the first time we have investigated the interaction of anticancer drug mitoxantrone with histone H1 and core histone proteins in solution using fluorescence, UV/Vis, CD spectroscopy and thermal denaturation techniques. The results showed that mitoxantrone reduced the absorbencies of H1 and core histone proteins at 210 nm (hypochromicity) and fluorescence emission intensity was decreased in a dose dependent. Binding of mitoxantrone changed secondary structures of the proteins as circular dichroism analysis confirmed it. Also, mitoxantrone increased the melting temperature of core histones at the final step of denaturation. The results suggest higher affinity of mitoxantrone to histone H1 compared to core histones providing histone proteins as a new target for mitoxantrone action at the chromatin level.

1. Introduction

Mitoxantrone is an anticancer drug, structurally related to anthracycline antibiotics, widely used in the treatment of various cancers such as breast cancer, lymphoma and leukemia [1,2]. It has a planar heterocyclic ring structure and the basic side groups (Fig. 1) critical for intercalation into DNA base pairs [3,4]. Binding of mitoxantrone to DNA inhibits both DNA replication and RNA transcription [5,6]. Also mitoxantrone is a potent inhibitor of topoisomerase II, an enzyme known to be important for the repair of damaged DNA and this leads to single and double strand breaks [7].

In the cell nucleus, DNA is compacted into chromatin, which is a complex structure built from DNA associated with basic proteins called histones [8,9]. There are 5 main histones: the linker histones of H1 family and core or nucleosomal histones H2A, H2B, H3 and H4. The core histones are small, basic proteins ranging between 11 and 16 kDa, with more than 20% of their amino acids composition being lysine and argentine [10]. Histone H1 is a very lysine-rich histone fraction of chromatin that binds to linker DNA between adjacent nucleosomes to facilitate the folding of the chromatin fiber [11].

To understand the exact mechanism of mitoxantrone action at the chromatin level, we have recently focused on the binding of mitoxantrone to chromatin and shown that the binding affinity of mitoxantrone to chromatin is higher than to DNA free of histones [12]. In the present study, we have extended our research on the effect of mitoxantrone on histone H1 and core histones and shown that most probably histone proteins play a fundamental role in drug–DNA interaction.

2. Materials and methods

2.1. Chemicals

Mitoxantrone hydrochloride was purchased from Helale Ahmar, Tehran, Iran (manufactured by Ebewe pharma Ges.m.b.Austria) stored at 4 °C in the dark. Before use, it was diluted to desired concentrations with 10 mM Tris–HCl (pH 7.2) and its concentration was determined spectrophotometrically using a molar extinction coefficient of 19,200 M^{-1} C m^{-1}. Histone H1 and core histones were prepared from calf thymus using 0.25 N HCl as described by Johns [13] and further purified [14]. The purity of the proteins was analyzed on SDS polyacrylamide gel electrophoresis as described by Laemmli [15]. The purified histones were dissolved in 10 mM Tris–HCl (pH 7.2) and after pH adjustment; the solution was stored at −20 °C and used within a month.

2.2. Drug treatment

Serial samples of histones were prepared individually (50–150 μg/ml) in 10 mM Tris–HCl (pH 7.2) and then appropriate concentrations of mitoxantrone (0–100 μM) were added and incubated for 40 min at room temperature in the dark. Free mitoxantrone, core histones and histone H1 (without the drug) were prepared in the same buffer and incubated along with the

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doi:10.1016/j.ijbiomac.2010.10.002

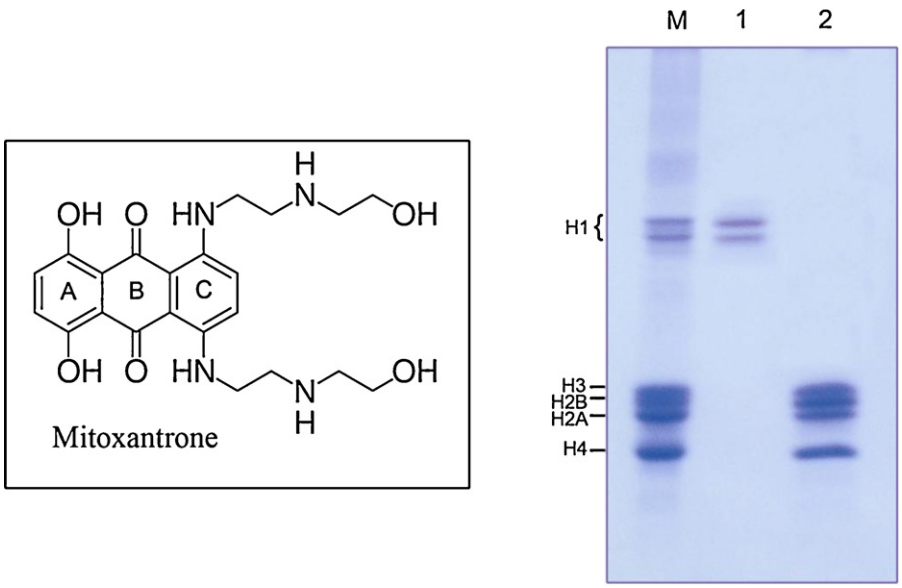

Fig. 1. Chemical formula of mitoxantrone and SDS-PAGE of histone proteins isolated from rat liver. 1, 2 are histone H1 and core histones, respectively. M is calf thymus histones as a marker.

drug-histone samples under the same experimental condition and used as a control.

2.3. Ultraviolet/visible spectroscopy

Core histones and histone H1 were mixed individually with mitoxantrone as described above in 10 mM Tris–HCl buffer (pH 7.2) and the spectrophotometric measurements were carried out at room temperature. The absorbencies were measured at multi λ system using a UV-160 Shimadzu spectrophotometer, equipped with quartz cuvettes. The changes in absorbencies were calculated by subtracting mitoxantrone absorbance at each point from that of protein–drug complex.

2.4. Fluorescence spectroscopy

The measurements were performed on a fluorescence spectrophotometer (Carry Eclipse-Bio-Varian model) using quartz cell of 1 cm × 1 cm path length. The mono chromatic slits were set at 5 nm to reduce the intensity of the signal depending on the experiment. All samples were made in 10 mM Tris–HCl (pH 7.2) at room temperature at a protein concentration of 50 μg/ml. After addition of the drug, the samples were allowed to equilibrate for 20 min before the fluorescence measurements were taken. Mitoxantrone and the histone solutions were prepared in the same buffer and used as a control. Amino acid tyrosine was also dissolved in the same buffer, and its emission spectrum was recorded in the same conditions and used as a reference. The spectra were recorded between 290 and 370 nm after excitation at 278 nm. The $I_0 - I/I_0 \times 100$ values were calculated for each sample and normalized with respect to the fluorescence of the protein in the absence of the drug in which I_0 and I are fluorescence intensity before and after the addition of mitoxantrone, respectively.

2.5. Circular dichroism (CD)

CD spectra were recorded on a CD spectrometer model 215 (AVIV instruments INC). The spectra were recorded from 190 to 260 nm (Far UV) using a 10 nm path-length cell. Various concentrations of mitoxantrone were added to histone solutions (200 μg/ml)

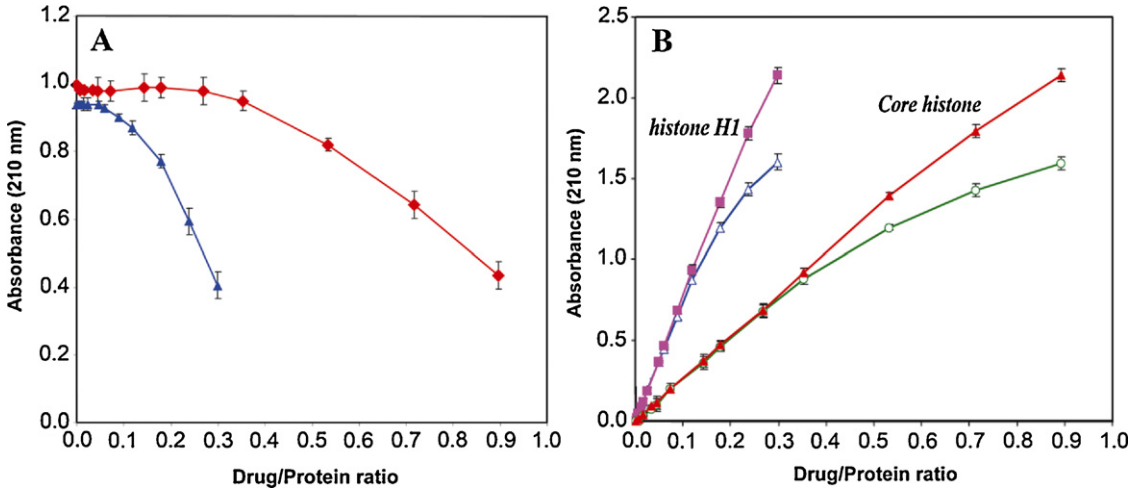

Fig. 2. Absorbance changes at 210 nm of histone proteins upon mitoxantrone binding. (A) Changes in the absorbance of histone H1 (▲) and core histones (♦) after subtraction of the drug absorbance. (B) Comparison of the absorbance of histone H1-mitoxantrone complex (△), sum of the absorbance of histone H1 and mitoxantrone (■) at 210 nm, absorbance of core histone–mitoxantrone complex (○), sum of the absorbance of core histone and mitoxantrone (▲). The results are presented as means ± SD.