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Spectroscopic detection of etoposide binding to chromatin components: The role of histone proteins

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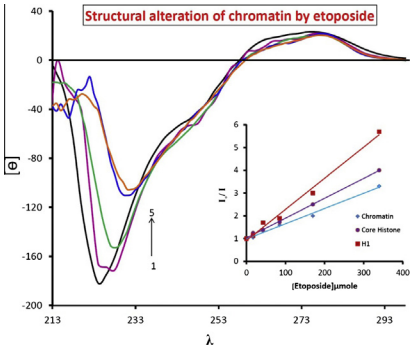
HIGHLIGHTS

- Structural alteration of chromatin upon etoposide binding.
- Higher affinity of etoposide to histone H1 compared to chromatin and DNA.
- Binding of etoposide to histone proteins increases the α-helical contents.
- Etoposide intercalated into DNA base pairs and quenches tyrosine residues of the histones.

GRAPHICAL ABSTRACT



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ABSTRACT

Chromatin has been introduced as a main target for most anticancer drugs. Etoposide is known as a topoisomerase II inhibitor, but its effect on chromatin components is unknown. This report, for the first time, describes the effect of etoposide on DNA, histones and DNA–histones complex in the structure of nucleosomes employing thermal denaturation, fluorescence, UV absorbance and circular dichroism spectroscopy techniques. The results showed that the binding of etoposide decreased UV absorbance and fluorescence emission intensity, altered secondary structure of chromatin and hypochromicity was occurred in thermal denaturation profiles. The drug exhibited higher affinity to chromatin compared to DNA. Quenching of drug chromophores with tyrosine residues of histones indicated that globular domain of histones is the site of etoposide binding. Moreover, the binding of etoposide to histones altered their secondary structure accompanied with hypochromicity revealing compaction of histones in the presence of the drug. From the results it is concludes that apart from topoisomerase II, chromatin components especially its protein moiety can be introduced as a new site of etoposide binding and histone proteins especially H1 play a fundamental role in this process and anticancer activity of etoposide.

Introduction

Etoposide is known as a potent anticancer drug to treat a variety of blood-born and solid malignancies [1]. The drug is a derivative of podophyllotoxin, a naturally occurring antimitotic agent from May apples, which contains a polycyclic ring system (ring A–D), a glycosidic moiety at C4, and a pendant E ring at C1 (Fig. 1). Etoposide exerts its biological action through binding to topoisomerase II and induces high level of transient protein-associated breaks [2,3]. It stabilizes a covalent enzyme cleaved DNA complex (known as the cleavage complex) that is a requisite intermediate in the catalytic cycle of topoisomerase II. The

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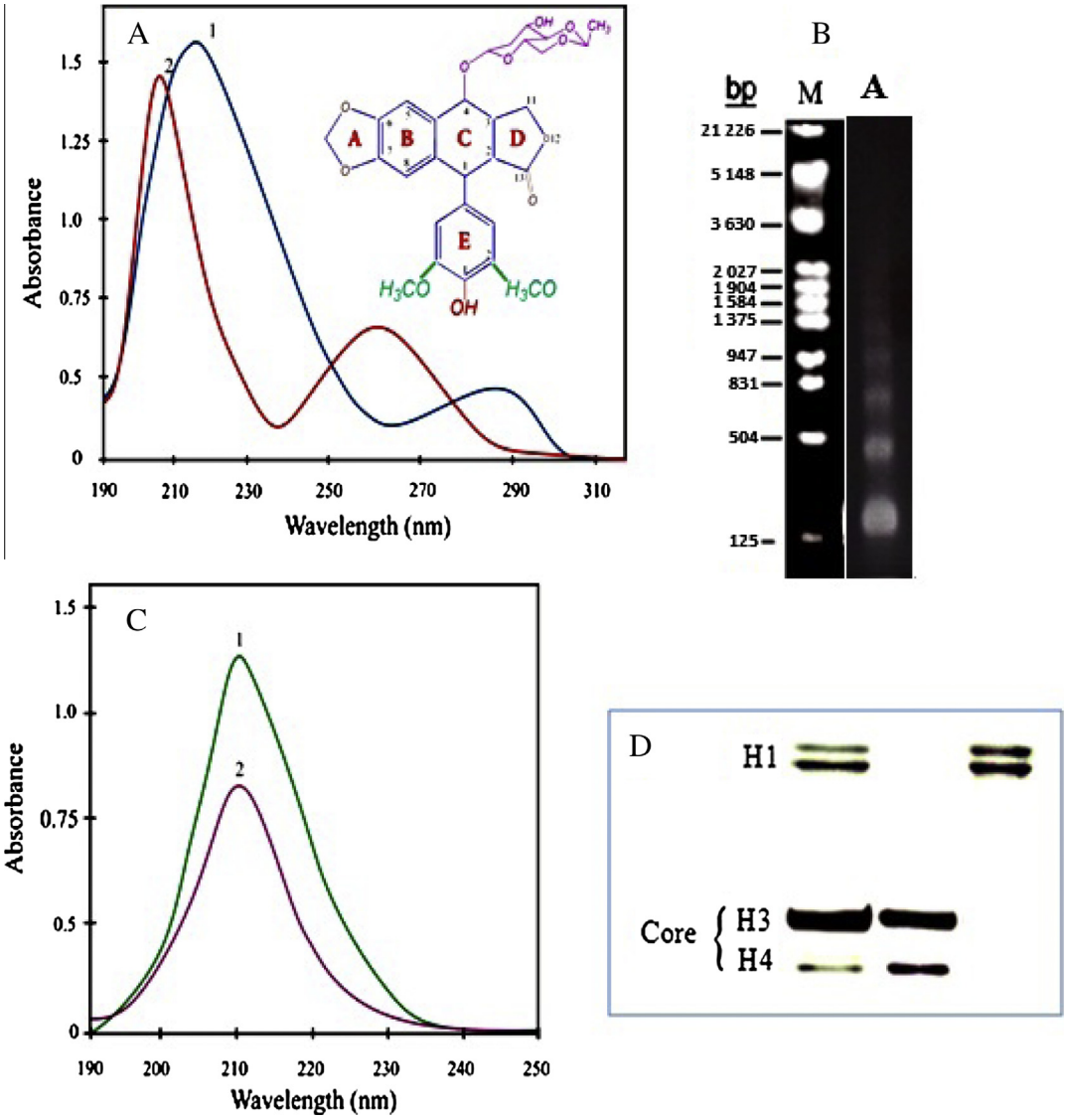

Fig. 1. (A) Chemical structure of etoposide and UV absorption spectrum of the drug (1) and chromatin (2) in 20 mM phosphate buffer (pH 7.4). (B) Agarose gel electrophoresis of soluble chromatin (A) and EcoRI Hind III digest DNA as a marker (M). (C) Spectra of core histone (1) and histone H1 (2). (D) Histone H1 and core histones analyzed on SDS PAGE and immunobloted against histones H1, H3 and H4 antibodies.

accumulation of cleavage complex in treated cells leads to generation of permanent breaks in the genetic materials which ultimately trigger cell death pathways [4–6]. Etoposide is a poor DNA intercalator, previous studies have indicated that because of nonpolar conformation adopted by etoposide, owing to the 1, 4 Trans arrangement of the E ring and glycosidic group, etoposide displays little ability to bind to free DNA [7]. It also induces apoptosis mainly through the cytochrome c/Apaf1/caspase 9 and Fas mediated death signaling pathways [8,9].

In the cell nucleus, DNA is packed into relatively small nucleoprotein particles named nucleosomes, the lowest level of chromatin organization [10]. In this structure, 146 bp of DNA is wrapped around octamer of histones particles H2A, H2B, H3, and H4. Each successive core is separated by linker DNA associated with a single molecule of histone H1. The core histones are small, basic proteins ranging between 11 and 16 kDa, with more than 25% of their amino acids composition being lysine and arginine. Histone H1 is a very lysine-rich histone fraction of chromatin with a molecular weight of 21 KDa and binds to linker DNA between adjacent nucleosomes to facilitate the folding of chromatin fiber [11,12].

Although, chromatin has been introduced as a main target for the most anticancer drugs, to date, no report has been published on the effect of etoposide on chromatin or its individual components. Therefore, in the present study the effect of etoposide on chromatin, histone proteins and DNA was investigated suggesting different binding affinity of the drug to DNA, chromatin and histone proteins.

Materials and methods

Etoposide (20 mg/ml) was purchased from 29-farvardin pharmacy, Tehran, Iran (manufactured by Teva Parenteral Medicines, Inc. Irvine, CA 92618) stored at 4 °C in the dark. Micrococcal nuclease (MNase) and calf thymus DNA were from Sigma Chemical Company (St. Louis, MO, USA). DNA was dissolved in 20 mM potassium phosphate buffer (pH 7.4), dialyzed overnight against the same buffer and its concentration determined using the molar extinction coefficient of 6600 $dm^3\ mol^{-1}\ cm^{-1}$ at 260 nm. All interactions were carried out in 20 mM potassium phosphate buffer (pH 7.4).