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ORIGINAL ARTICLE

Differential effects of anti-cancer and anti-hepatitis drugs on liver cystatin

Aaliya Shah [a], Medha Priyadarshini [b], Mohd Shahnawaz Khan [c], Mohammad Aatif [d], Fakhra Amin [d], Shams Tabrez [e], Galila F. Zaher [f], Bilqees Bano [d,*]

[a] *Department of Biochemistry, SKIMS Medical College, Srinagar, India*
[b] *Department of Medicine, Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA*
[c] *Department of Biochemistry, Protein Research Chair, College of Science, King Saud University, Riyadh, Saudi Arabia*
[d] *Department of Biochemistry, Faculty of Life Sciences, AMU, Aligarh, India*
[e] *King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia*
[f] *Department of Haematology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

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KEYWORDS

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Fluorescence;
UV-spectroscopy

Abstract The drug–protein interaction has been the subject of increasing interest over the decades. In the present communication, the interaction of liver cystatin with anti-cancer (adriamycin) and anti-hepatitis (adevofir dipivoxil) drugs was studied by thiol-protease inhibitory assay, UV absorption, fluorescence spectroscopy and circular dichroism (CD). A static type of quenching was observed between the protein and the drug molecules. Binding constant (Ka) of adriamycin to liver cystatin (LC) was found to be 1.08×10^6 M^{-1}. Moreover, binding site number was found to be 2. Importantly, cystatin loses its activity in the presence of adriamycin. However, intrinsic fluorescence studies in the presence of adevofir dipivoxil showed enhancement in the fluorescence intensity suggesting that binding of adevofir to LC caused unfolding of the protein. The unfolding of the test protein was also accompanied by significant loss of inhibitory activity. CD spectroscopy result showed, both adriamycin and adevofir dipivoxil caused perturbation in the secondary structure of liver cystatin. The possible implications of these results will help in combating drug induced off target effects.

Abbreviations: LC, liver cystatin; ADR, adriamycin; CD, circular dichroism; Ka, binding constant; HBV, human hepatitis B virus.

* Corresponding author.
E-mail address: bbano08@rediffmail.com (B. Bano).

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1. Introduction

Drug–protein associations are vital, since most of the administered drugs are reversibly bound to proteins. The bound drugs are transported mainly as a complex with these proteins. The binding factors are useful in studying the pharmacological response and drugs dosage design (Borga and Borga, 1997).

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The present report summarizes the interaction of goat liver cystatin (thiol-protease inhibitor) with an anti-sarcoma drug, adriamycin and an anti-hepatitis drug, adevofir dipivoxil. Adriamycin (doxorubicin hydrochloride) is an excellent anti-tumor antibiotic and is very effective against a large number of human malignancies. The anti-cancer activity of adriamycin is associated with the formation of intercalative complexes with DNA (Bryn and Dolch, 1978).

Adevofir dipivoxil is a diester prodrug of adevofir. It is an acyclic nucleotide analog having activity against human hepatitis B virus (HBV). Moreover, it inhibits HBV-DNA polymerase (reverse transcriptase) action via natural substrate deoxyadenosine triphosphate binding and DNA chain termination. The chemical structure of adriamycin (doxorubicin hydrochloride) and adevofir dipivoxil is shown in Figs. 1 and 2, respectively.

Cystatins are the family of proteins that regulate and inhibit the detrimental effect associated with cysteine proteases (Ekiel et al., 1997). Cystatins could protect the cells from unnecessary proteolysis which might lead to several pathological conditions (Shah and Bano, 2009).

The goat liver cystatin used in the present study was purified in our laboratory (Shah and Bano, 2011). Further, conformational changes in the purified thiol protease inhibitor after association with anti-cancer and anti hepatitis drugs were monitored by UV–visible, fluorescence and circular dichroism spectroscopic techniques. Moreover, the current paper also addresses the kind of interaction involved in the binding of these drugs with thiol protease inhibitor.

2. Materials and methods

2.1. Materials

Casein, papain, EDTA, acetone, sephacryl-S100HR, CBB R-250 and cysteine were procured from Sigma Aldrich. Adriamycin (doxorubicin hydrochloride) was purchased from VHB Life Sciences Limited India. Adevofir dipivoxil was purchased from Sun Pharmaceutical Industries, India. All other chemicals used were of analytical grade.

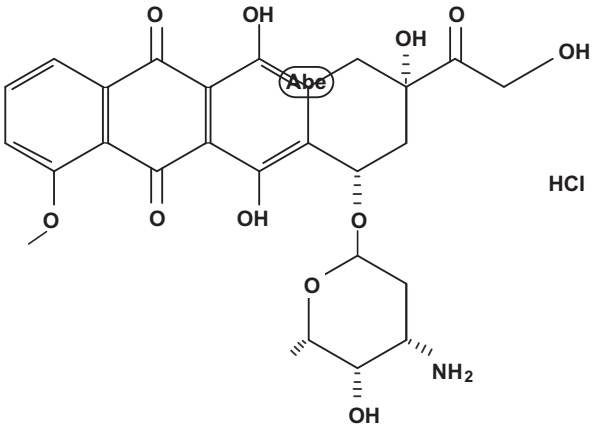

Figure 1 Chemical structure of adriamycin (doxorubicin hydrochloride).

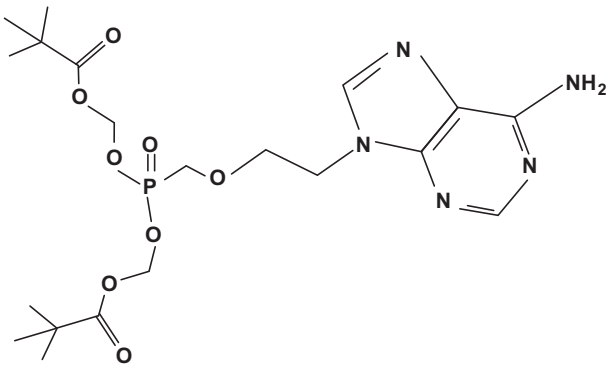

Figure 2 Chemical structure of adevofir dipivoxil.

2.2. Methods

2.2.1. Protein estimation

The concentration of purified protein was quantitated by the Lowry et al. (1951) method.

2.2.2. Preparation of drug solutions

As adriamycin (ADR) is sensitive to light and oxygen, a stock solution of ADR within the therapeutic range in normal saline was prepared just before use. 2 μM of goat LC was incubated with varying concentrations of ADR in the range of 0.5–3 μM for 30 min. Moreover, a stock solution of adevofir dipivoxil in 0.05 M sodium phosphate buffer (pH 7.2) was prepared fresh just before use. Goat liver cystatin at a concentration of 2 μM was incubated with varying concentrations of adevofir dipivoxil (0.1–1 μM) for 30 min.

2.2.3. Thiol protease inhibitory activity assay

Aliquots from the incubated samples were tested for their thiol protease inhibition potential by the method of Kunitz (1947).

2.2.4. UV–Visible spectroscopy

Absorption spectra of cystatin and cystatins incubated with ADR and adevofir dipivoxil were measured on a UV–visible spectrophotometer at 220–400 nm wavelength range by the use of 1 cm path length cell holder.

2.2.5. Fluorescence spectroscopy

The measurements of fluorescence were recorded on a spectrofluorometer (Shimadzu) at 25 °C by the use of a quartz cell of 1 cm path length. The fluorescence of cystatin bound drugs was recorded at the wavelength range of 250–400 nm after exciting the complex at 280 nm.

2.2.6. Circular dichroism measurement

Far-UV CD measurements were recorded by the use of a circular dichroismchiroptical spectrometer (Applied Photophysics, Chira-scan-Plus, UK). Samples were maintained at 25 °C with the help of circulating water bath in a 1 mm quartz cuvette. Spectra of LC in the absence and presence of various concentrations of adriamycin and adevofir dipivoxil were measured in the range 190–250 nm with a step size of 1.0 nm.