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Exploring binding affinity of oxaliplatin and carboplatin, to nucleoprotein structure of chromatin: Spectroscopic study and histone proteins as a target

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ABSTRACT

Platinum drugs are potent chemotherapeutic agents widely used in cancer therapy. They exert their biological activity by binding to DNA, producing DNA adducts; however, in the cell nucleus, DNA is complexed with histone proteins into a nucleoprotein structure known as chromatin. The aim of this study was to explore the binding affinity of oxaliplatin and carboplatin to chromatin using spectroscopic as well as thermal denaturation and equilibrium dialysis techniques. The results showed that the drugs quenched with chromophores of chromatin and the quenching effect for oxaliplatin (Ksv = 3.156) was higher than carboplatin (Ksv = 0.28). The binding of the drugs exhibited hypochromicity both in thermal denaturation profiles and UV absorbance at 210 nm. The binding was positive cooperation with spontaneous reaction and oxaliplatin ($Ka = 5.3 \times 10^3\ M^{-1}$, $n = 1.7$) exhibited higher binding constant and number of binding sites than carboplatin ($Ka = 0.33 \times 10^3\ M^{-1}$, $n = 1.0$) upon binding to chromatin. Also secondary structure of chromatin proteins was altered upon drugs binding. It is concluded that oxaliplatin represents higher binding affinity to chromatin compared to carboplatin. In chromatin where DNA is compacted into nucleosomes structure with histones, the affinity of the platinated drugs is reduced and histone proteins may play a fundamental role in this binding process.

1. 1ntroduction

Platinum anticancer drugs are inorganic heavy metal complexes currently used in clinical treatment of various types of cancer including colorectal, non-small-cell lung, and genitourinary cancers [1,2]. Since the discovery of therapeutic potential of cisplatin, a large number of platinum analogs have been synthesized and some of them have shown anticancer properties [3]. Carboplatin and oxaliplatin (Fig. 1A) are second and third generation platinum drugs with a wide spectrum of antitumor activity, a lower toxicity than their parent molecule, cisplatin, and capability of invading cisplatin resistance [4,5].

Upon entrance into cells, all platinum drugs become activated by aquation, losing chloride, 1,1-cyclobutane dicarboxylae or oxalate ions, and gain two water molecules. They bind to N7 atom of imidazole ring of guanine residues and form interstrand and intrastrand DNA cross-links between platinum and nucleotides, called DNA–platinum adducts [6,7]. Oxaliplatin shows lower reactivity than cisplatin with naked DNA and cellular DNA. It has been reported that in some cell lines, level of platinum-DNA adducts and intrastrand cross-links are lower for oxaliplatin than for cisplatin [8,9]. These changes in DNA structure arrest DNA replication and transcription, triggering a variety of responses, which lead to cell death [10].

It is generally believed that DNA is the preferential and cytotoxic target for platinated agents [3,6] but in the cell nucleus DNA is as a supramolecular nucleoprotein structure, known as chromatin. The nucleosomes, repeating unit of chromatin, represent a structure formed by wrapping of 145 bp DNA around an octamer of core histone consisting of two H2A/H2B dimers and a central H3/H4 tetramer. Histone H1 binds to linker DNA connecting adjacent nucleosomes and is responsible of the higher order structure of chromatin [11,12]. Thus, chromatin, but not DNA, is the major target for platinum anticancer drugs *in vivo*. Although binding of cisplatin to nucleosomes structure and reconstitutes chromatin has been studied in detail [13,14], no work has been published on the binding affinity of oxaliplatin and carboplatin to chromatin structure. Therefore how chromatin is affected by binding of these drugs is an important question that needs to be elucidated for a better understanding of the molecular mechanism of platinum-based drugs

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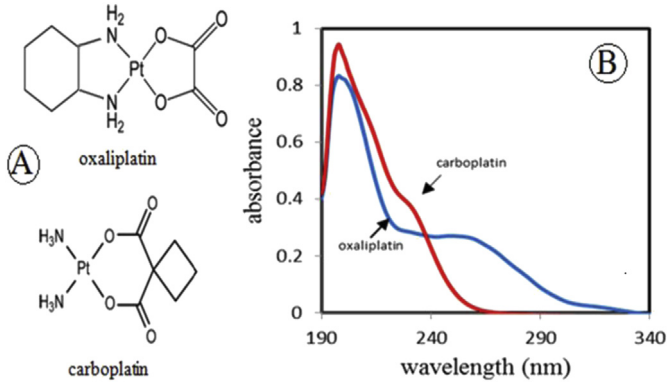

Fig. 1. Chemical formula (A) and UV absorption spectrum of oxaliplatin and carboplatin (B) in 20 mM phosphate buffer (pH 7.2).

action. In the present study, we have tried to explain mechanism of the interaction between chromatin and platinum drugs, oxaliplatin and carboplatin and the results demonstrate higher affinity of oxaliplatin to chromatin compared to carboplatin. Also, histone proteins may play a role in this binding process.

2. Materials and methods

Oxaliplatin (5 mg/mL) and carboplatin (10 mg/mL) were purchased from Helale Ahmar, Tehran, Iran (manufactured by Aventis Pharma Dagenham and Koak, Farma, respectively). The drugs were diluted with 20 mM sodium phosphate buffer (pH 7.2) at a concentration of 1 mg/mL and stored at −20 °C in the dark. Micrococcal nuclease (MNase) was from Sigma Chemical Company.

Albino rats weighing 150–200 g of either sex were used throughout the experiments. They were obtained from the Animal Center of Institute of Biochemistry and Biophysics Tehran, Iran. They were maintained in conventional pathogen free conditions in a temperature (22 °C–23 °C), humidity (50%–70%), and photoperiod of 12 h dark/light cycle controlled room.

2.1. Preparation of rat liver chromatin

Soluble chromatin was prepared from rat liver nuclei according to the procedure reported previously [15] with some modifications. Briefly, the purified, intact nuclei were suspended in digestion buffer composed of 0.25 M sucrose, 25 mM NaCl, 1 mM $CaCl_2$ and 10 mM Tris–HCl (pH 7.4) and DNA content determined assuming that the absorbance of 1 mg/mL of DNA at 260 nm is 20. The nuclear suspension was digested with micrococcal nuclease (4 units/mg of DNA) for 10 min at 37 °C. The solution was then brought to 10 mM EDTA and centrifuged at 8000 g for 5 min. The pellet was resuspended in 0.25 mM EDTA (pH 7.5) and the nuclei lysed by gentle stirring at 4 °C for 1 h. The lysate was then centrifuged at 8000 g for 10 min and the chromatin in the supernatant was designated as soluble chromatin.

2.2. UV/vis spectroscopy

The soluble chromatin (50 μg/mL) was incubated in the absence and presence of various concentrations of oxaliplatin or carboplatin in 20 mM sodium phosphate buffer (pH 7.2) at room temperature in the dark for 1 h. Various concentrations of the drugs (in the absence of chromatin) were also incubated along with the treated samples under the same experimented condition and uses as a reference. Spectroscopic analysis of drug treated and the controls were made on a Shimadzu spectrophotometer (model UV-260) equipped with temperature controller using quartz cells with path length of 1 cm. Difference spectra were drawn for chromatin–drug complexes between 190 and 300 nm against various concentrations of oxaliplatin or carboplatin in the same buffer.

2.3. Thermal denaturation analysis

Thermal denaturation measurement of chromatin (50 μg/mL), in the absence and presence of the drugs was carried out by stoppered quartz cuvettes on a Carry 100 Bio UV–vis spectrophotometer. The samples were continuously heated at 1 °C/min and absorbance changes at 260 nm monitored using the same drug concentration in the reference sample to minimize absorbance of the drug at this wavelength. Corresponding derivative denaturation profiles were drawn using Cary Win UV software using equation $dh(T)/dT = h(T+1) - h(T-1)/2$, where h is hyperchromicity, T is temperature and dh denotes derivative of hyperchromicity [16].

2.4. Fluorescence spectroscopy

Fluorescence measurements were performed on a Cary Eclipse (Varian) fluorescence spectrophotometer equipped with a thermostatically controlled cell holder at ambient temperature. The excitation and emission wavelengths were set at 278 nm and 295–395 nm, with the excitation and emission slit widths of 5 and 10 nm, respectively, to reduce the intensity of the signal depending on experiment. Fluorescence emission intensity of chromatin was measured in the absence and presence of various concentrations of the drugs (0–180 μg/mL) using quartz cells of 1 cm path length. $(I_o - I/I_o \times 100)$ values was estimated and normalized with respect to the fluorescence of the samples in the absence of the drugs in which I_o and I are fluorescence emission intensity before and after addition of the drugs, respectively. Fluorescence quenching data were analyzed in term of Stern–Volmer constant, Ksv, using $I_o/I = 1 + Ksv\,[Q]$ where Q is molar concentration of quencher (oxaliplatin or carboplatin). Ksv was obtained from the slope of I_o/I versus [Q] plot [17]. The association constant (K_a) and number of binding sites (n) were determined from the slope and intercept of the modified Stern–Volmer plot $\log [I_o - I/I] = \log Ka + n \log [Q]$. Thermodynamic parameters of the binding were estimated from Van't Hoff equation $\ln K = -\Delta H/RT + \Delta S/R$, where K is the binding constant at the corresponding temperature (T), and R is the gas constant. Also, free energy change (ΔG) was determined using $\Delta G = \Delta H - T\Delta S$ [18,19].

2.5. Equilibrium dialysis

Chromatin (50 μg/mL) was prepared in 20 mM phosphate buffer (pH 7.2) and dialyzed against the same buffer containing serial concentrations of oxaliplatin or carboplatin using Spectrum laboratories dialysis tubing at (23 °C). The equilibrium was achieved within 72 h. The total drug concentrations (C_t) and concentrations of free drug (C_f) were determined using extinction coefficient of 6600 M^{-1} cm^{-1} for DNA [20]. Extinction coefficient of the drugs (ε_{210}) was estimation from the slope of the absorbance versus drugs concentration using $A = \varepsilon$ cl [16] and designated as 4513 M^{-1} cm^{-1} for oxaliplatin and 3370 M^{-1} cm^{-1} for carboplatin. The amount of bound drug (C_b) was obtained from $C_b = C_t - C_f$. Binding parameters were calculated from the plot of r/C_f versus r according to Scatchard method where r is the molar concentration of bound drug. Scatchard plot gives an x-intercept of n, where n is the apparent number of binding sites and K_a (apparent binding constant) [21]. Hill coefficient (n_H) was determined from the slope of $\ln (C_f)$ versus $\ln (r/n - r)$ according to Hill equation [16].