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Platinum drugs binding to human serum albumin: Effect of non-steroidal anti-inflammatory drugs

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ABSTRACT

In this report, we provide investigations about the effect of Sudlow's site I and II blockers on the platinum anticancer drugs binding. The effect of warfarin, aspirin, ibuprofen and meloxicam on the platinum binding to HSA has been investigated through gel-filtration chromatography, UV–vis, CD, fluorescence spectroscopy and the inductively coupled plasma atomic emission spectroscopy (ICP(AES)) method.

The results confirm that the platinum complexes bind to Sudlow's site I and II. Modifications of the protein structure caused by non-steroidal anti-inflammatory drugs (NSAIDs) that bind in these areas have an impact on the amount of cisplatin bound to albumin. The number of bound cisplatin was reduced by approximately 30–40% compared to the native protein.

1. Introduction

Platinum complexes are successfully used in clinical practice for the treatment of several solid tumors. Platinum-containing anticancer drugs are believed to induce apoptosis in cancer cells by covalently binding to DNA [1,2] however, they also react with a number of proteins and peptides.

Cis-diamminedichloroplatinum(II) (cisplatin), is one of the leading drugs currently used in the treatment of a number of solid tumors. Owing to its serious side-effects, attempts have been made to develop less toxic analogs of cisplatin that are just as clinically effective. The new generation of Pt-complexes (carboplatin, oxaliplatin, nedaplatin, lobaplatin), still exhibit a substantial level of unspecific toxicity and low activity against many widespread cancers and metastases [3–5].

Today cisplatin is one of the most widely used anticancer drugs, together with the second generation drug carboplatin. These drugs are particularly effective in combination chemotherapy [6,7].

Protein interaction with cisplatin studies indicated that cisplatin is believed to be therapeutically active only when it is not bound to proteins [8]. Cisplatin is administered intravenously, and within 1 day, 65–98% of the drug is bound to blood plasma proteins particularly albumin [9]. It has been postulated that in addition to drug inactivation, cisplatin binding to proteins may be the cause of many of the drug's side-effects. Cisplatin and its analog carboplatin (CPT), $[Pt(NH_3)_2(cbdca)]$ (cbdca is 1,1-cyclobutanedicarboxylate), are an important chemotherapeutic drugs for cancer treatment, however, the modes of action and toxicity of these drugs are not well understood. Both of the drugs have identical mechanism of action, based on selective DNA platination. The essential difference between investigated platinum complexes is a kinetic one. It is known that replacement of two chloride groups by the bulkier cbdca bidentate ligand, while not changing the overall charge of the complex, renders the aquation process of CPT far slower than in cisplatin.

The information on how platinum drugs interact with proteins is important for the understanding of the mechanisms of action and toxicity of a drug and the optimization of cancer treatments.

Human serum albumin (HSA) plays an important role in the transport and disposition of endogenous and exogenous ligands present in blood [10,11]. In general, more than 90% of the drugs used in humans are bound to this specific protein. HSA a single-chain protein, is composed of three homologous, predominantly helical domains (I–III); each of these is comprised of two subdomains A and B. At physiological pH, albumin adopts helical conformation (67%-helix), and its amino acid sequence contains 17 disulfide groups, one thiol group (cysteine-34), and one tryptophan residue (tryptophan-214). The principal regions of ligand binding sites of albumin are located in hydrophobic cavities in subdomains IIA

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(Sudlow's site I) and IIIA (Sudlow's site II), which exhibit similar chemistry [12,13].

Most non-steroidal anti-inflammatory drugs (NSAIDs) used during cancer therapy, show a high degree of binding to albumin, which is a primary determinant of their pharmacokinetic properties. Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) is pharmacologically important new generation, non-steroidal anti-inflammatory drug of enolic acid class compounds [14]. The inhibition of the prostaglandin synthesis through the blockade of cyclooxygenase (COX) has been widely accepted as the mechanism of action of the so-called coxibs. The meloxicam makes selective inhibition to COX-2 more than COX-1 [15,16]. The primary function of these drugs is anti-inflammatory effect but they can also be used as agents in cancer treatment, because in various types of cancer, COX-2 is over expressed [17]. COX-derived prostaglandins (PGs) have been shown to modulate cell proliferation, apoptosis, angiogenesis, and immune surveillance [18,19]. The plasma protein binding of meloxicam is more than 99% [16].

Our earlier studies [20] have shown that meloxicam causes destabilization of the Sudlow's site I located in subdomain IIA of albumin molecule. The meloxicam binding site was compared with aspirin which is the most popular traditional non-steroidal anti-inflammatory drug administered together with a new generation of drugs. As previously reported [21] aspirin binds with albumin in two, IIA and IIIA, subdomains. Usually, drugs bind to high-affinity sites with typical association constants in the range of 10^4–10^6 M^{-1}. Thus, the binding of particular drug molecule to serum albumin may change considerably binding abilities of HSA toward other molecules.

Reaction between cisplatin and serum albumin is thought to be the main route for platinum binding in human blood plasma [10]. Cisplatin binding to albumin is essentially irreversible with less than 5% loss of protein-bound platinum after extensive dialysis. Previous studies of cisplatin binding to HSA [22–25] have emphasized the important role of the softer cysteine and methionine sulfur atoms as preferred targets. It is interesting to note that transplatin (trans-diamminedichloroplatinum(II)), the closest structural isomer of cisplatin, exhibits notably higher disruption reactivity than cisplatin.

As suggested by earlier study [26], platinum complexes can be bound to albumin in the subdomain IIA (Sudlow's site I) causing the district modification of HSA native structure, and affect the affinity of albumin toward endogenous ligands such as heme and bilirubin.

Numerous studies show that the hydrophobic cavities in subdomains IIA is also binding site a number of other drugs [12,20] including used as analgesics and chemopreventive agents, non-steroidal anti-inflammatory drugs [27–30].

In this report, we provide investigations about the effect of meloxicam, aspirin, ibuprofen and additionally warfarin (as a marker for Sudlow's site I) on the platinum anticancer drugs binding.

Studies using ligands which the binding sites in albumin molecule have been well defined may be helpful in more accurate identification of the platinum drugs binding. It should be noted that in spite of many years of research on the binding of platinum complexes with albumin, precise identification of all binding sites was not possible.

2. Materials and methods

2.1. Materials

High purity HSA >98% (GE), cis-DDP (cis-dichlorodiammineplatinum(II)), trans-DDP (trans-dichlorodiamineplatinum(II)) and warfarin (3-(α-acetonylbenzyl)-4-hydroxycoumarin) were obtained from Sigma–Aldrich. Carboplatin was purchased from Strem Chemicals. Meloxicam was supplied from Alfa Aesar. ANS, dansylsarcosine piperidinium salt and ibuprofen sodium salt (α-methyl-4(isobutyl)phenylacetic acid sodium salt) were supplied by Sigma–Aldrich. Aspirin was purchased from Ubichem.

The stock solution of meloxicam was prepared by dissolving in minimal amounts of methanol, diluted with double distilled water and used in all experiments from a freshly prepared 0.2 mM solutions (the maximum methanol content did not exceed 10%, v/v). In the final step a stock solution was diluted with the buffer and added dropwise to the protein solution.

HSA concentration was determined by absorption spectrum, taking the absorbance of a 1 mg/cm^3 solution at 280 nm as 0.55 [31]. The HSA–platinum (II) complexes were prepared by incubation of the reaction mixtures for 24 and 48 h at 37 °C in sterilized tubes.

In all of the experiments, a sodium phosphate buffer (0.05 M, pH 7.4) containing 0.1 M NaCl was used.

2.2. Methods

The circular dichroism (CD) spectra were determined on a Jasco J-715 spectropolarimeter over the range of 190–250 nm using a 0.1 cm quartz cell. The spectra are expressed as MRE (mean residue ellipticity, θ_{MRE}) in deg cm^2 dmol^{-1}. HSA (8×10^{-6} M) was incubated with platinum(II) complexes (8×10^{-5} M) for 24 h at 37 °C pH 7.4 (0.05 M sodium phosphate buffer, containing 0.1 M NaCl).

Fluorescence measurements were carried out on an SLM AMINco SPF-500 spectrofluorimeter and 1 cm × 1 cm × 4 cm quartz cells. Tryptophan fluorescence of albumin was measured by exciting the protein solution at 298 nm. The emission spectra were recorded from 300 to 500 nm. Warfarin-containing samples were measured by exciting at 335 nm and the emission wavelength maximum was found at 378 nm. ANS was measured by exciting at 370 nm and emission wavelength at 470 nm. Warfarin and ANS were used as site marker fluorescence probes for monitoring sites I of HSA.

Blank values corresponding to the buffer were subtracted to correct for the background fluorescence.

In fluorescence experiments, HSA concentration was 4×10^{-5} M. HSA was incubated with platinum(II) complexes for 24 h at 37 °C pH 7.4 (0.05 M sodium phosphate buffer, containing 0.1 M NaCl). Molar ratio Pt(II)/HSA = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10.

The assays of platinum(II) bounded per mol of HSA were performed with a 3410 ICP(AES) spectrometer. HSA was allowed to react with a 20-fold molar excess of platinum for 2 days at pH 7.4 at 37 °C after saturation by blocking agents (warfarin, meloxicam, aspirin and ibuprofen). Molar ratio drug/protein was 2/1. Concentration of HSA was 1.25×10^{-4} M. The platinum–HSA complexes were separated by gel filtration on a Sephadex G-75 column. The platinum content in a selected fraction was determined by the ICP(AES) method. Protein concentration measurements were determined spectrophotometrically at 280 nm (spectrometer UV-Vis Perkin Elmer Lambda 20).

3. Results and discussion

3.1. Impact of Pt(II) complexes on the structure of HSA

HSA consists predominantly of α-helices. Approximately, 67% of HSA is helical, the number of helices in the structure is 28 [11]. CD spectra of HSA exhibit two negative bands in the ultraviolet