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Studies of drug interactions with alpha$_1$-acid glycoprotein by using on-line immunoextraction and high-performance affinity chromatography

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ABSTRACT

A method that combined on-line immunoextraction with high-performance affinity chromatography was developed to examine the binding of drugs with α_1-acid glycoprotein (AGP). Affinity microcolumns containing immobilized polyclonal anti-AGP antibodies were developed that had a capture efficiency of up to 98.4% for AGP and a binding capacity of 0.72 nmol AGP when using a 20 mm × 2.1 mm i.d. microcolumn. These microcolumns were employed in various formats to examine the binding of drugs to normal AGP and AGP that had been adsorbed from serum samples for patients with systemic lupus erythematosus (SLE). Drugs that were screened in zonal elution experiments for their overall binding to these types of AGP included chlorpromazine, disopyramide, imipramine, propranolol, and warfarin. Most of these drugs showed an increase in their binding to the AGP from SLE serum when compared to normal AGP (i.e., an increase of 13–76%); however, disopyramide gave a 21–25% decrease in retention when the same AGP samples were compared. Frontal analysis was used to further evaluate the binding of disopyramide and imipramine to these forms of AGP. Both drugs gave a good fit to a model that involved a combination of saturable and non-saturable interactions with AGP. Changes in the non-saturable interactions accounted for most of variations seen in the binding of disopyramide and imipramine with the AGP samples. The methods used in this study could be adapted for use in personalized medicine and the study of other proteins or drugs using aqueous mixtures or clinical samples.

1. Introduction

The binding of drugs with serum proteins is important in determining the transport, excretion and metabolism of many pharmaceuticals in the body [1–3]. Alpha$_1$-acid glycoprotein (AGP) is a transport protein that is often involved in this process, with this protein binding to numerous basic and neutral drugs in blood [4–6]. Human AGP has an average molecular weight of 41 kDa and a normal serum concentration that ranges from 0.5–1.0 mg/mL [1,4,7]. However, the concentration of AGP can be altered in response to infection, trauma, acute illness, chronic inflammatory disorders, and diseases such as systemic lupus erythematosus (SLE) [4,5]. In addition, AGP has a high carbohydrate content (>45% of the total weight of this protein), and modifications in its glycan chains have been observed in conditions such as rheumatoid arthritis, SLE, and some types of cancer [5,8,9].

SLE is a severe, chronic autoimmune disease in which the body's immune system attacks healthy tissues [9–11]. This is a highly heterogeneous condition that can affect the skin, joints, kidneys, brain, and other organs [11,12]. The onset of SLE can occur at any age; however, the incidence of this disease is highest in young women of childbearing age [11–13]. Previous studies have noted high plasma levels of AGP in patients with SLE [5]. In addition, a differential glycosylation pattern of AGP (i.e., an increase in bi-antennary glycans, particularly in patients who have accompanying infections) has been reported in SLE [5,9]. This suggests that the binding of some drugs with AGP may change during SLE due to the variation in the concentration of this protein or its glycoform pattern. However, little information exists on how the drug-binding properties of AGP may occur under these conditions.

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There are several approaches that have been developed for the analysis of drug-interactions with proteins like AGP. These approaches have included separation methods such as equilibrium dialysis, ultrafiltration, liquid chromatography, and capillary electrophoresis (CE), and non-separation methods such as absorption or fluorescence spectroscopy, calorimetry, and surface plasmon resonance spectroscopy [3,14–19]. High-performance affinity chromatography (HPAC) is a separation method that has been used in various studies to examine drug-protein binding [1–3]. This technique utilizes a biologically-related ligand as the stationary phase and has been widely applied to the examination of drug-protein interactions by using columns that contain immobilized proteins like AGP or human serum albumin (HSA) [1,2,20]. Advantages of this method include its selectivity, speed, precision, ease of automation, and ability to work with small amounts of a target solute or binding agent [1,2,20].

When HPAC is employed for studying drug-protein interactions, an important factor to consider is the selection of the immobilization method to place the binding agent in the column. Several methods have been developed to immobilize AGP in HPAC columns [21–23]. For example, a covalent immobilization method has been described in which mildly-oxidized AGP is attached to hydrazide-activated silica [21]. Entrapment has also been employed, in which the physical containment of a protein such as AGP has been accomplished by using a hydrazide-activated support that is capped with oxidized glycogen [23]. However, these immobilization methods work best with a highly pure binding agent and cannot be used directly with samples that contain complex mixtures of proteins, as occurs in serum [14].

On-line immunoextraction is an alternative method for column preparation that can be used to both isolate and immobilize a binding agent that is present in a complex sample like serum [24,25]. Immunoextraction is a technique that utilizes immobilized antibodies against a given target to capture and isolate this target from a sample [24–26. Recently,a method combining on-line immunoextraction with HPAC has been developed and shown to be a rapid means for examining drug interactions with the major serum protein HSA [24]. In this prior work, polyclonal antibodies against HSA were placed into a column, followed by the adsorption of this protein to the antibodies and the use of this adsorbed protein in drug binding studies, as is illustrated in Fig. 1 [24,26].

This report will examine the extension and modification of on-line immunoextraction and HPAC to examine the interactions of drugs with a relatively low concentration protein, such as AGP that has been isolated from normal serum or serum from individuals with SLE. Both zonal elution and frontal analysis experiments will be conducted on the adsorbed samples of AGP and by using various drugs that are known to interact with this protein. The information obtained through this work should provide more detailed information on how the interactions of AGP with drugs may change during SLE. These results should make it possible to determine the advantages of using on-line immunoextraction and HPAC with relatively low concentration proteins like AGP for drug-binding studies. The same method should also be applicable to other types of drug-protein binding studies and in work with additional disease states, as could be used in clinical studies or for personalized medicine [24].

2. Experimental section

2.1. Materials

The chlorpromazine (≥98% pure), *R/S*-disopyramide (≥98%), imipramine (≥99%), *R/S*-propranolol (≥99%), *R/S*-warfarin (≥98%), AGP (from pooled human plasma, ≥99%, product no. G9885), goat immunoglobulin G (IgG; reagent grade, ≥95% purity, lyophilized; product no. I5256), human serum (from pooled male AB plasma, sterile-filtered, product no. H4522), poly(ethylene oxide) (PEO; average molar mass, 8000 kDa), Brij 35 (average molar mass, 1.198 kDa), and Lucifer yellow CH (LyCH, dilithium salt) were from Sigma-Aldrich (St. Louis, MO, USA). The polyclonal anti-human AGP antibodies (goat anti-AGP, affinity-purified, catalog no. GAGP-80A) were obtained from Lee Biosolutions (Maryland Heights, MO, USA). De-identified and pre-existing serum samples from patients with SLE were provided by W. Clarke (Note: this work was determined to be exempt from IRB review by the Johns Hopkins School of Medicine, according to the Code of Federal Regulations – 45 CFR 46.101 b). The Nucleosil Si-1000 (7 μm particle diameter, 1000 Å pore size) was from Macherey Nagel (Düren, Germany). Reagents for the micro bicinchoninic (BCA) protein assay were purchased from Pierce (Rockford, IL, USA). All other chemicals were of the purist grades available. All aqueous solutions were prepared using water from a Milli-Q Advantage A10 system (Millipore, Billerica, MA, USA) and were filtered through 0.20 μm GNWP nylon membranes from Millipore. The Zeba spin desalting columns (0.5 mL, 7 kDa cutoff) were from Thermo Fisher Scientific (Rockford, IL, USA).

2.2. Apparatus

The affinity microcolumns were packed using an HPLC slurry packer from ChromTech (Apple Valley, MN, USA). The chromatographic system consisted of a DG-2080-53 degasser, two PU-2080 Plus pumps, an AS-2057 Plus autosampler, a CO-2067 Plus column oven, and a UV-2075 absorbance detector from Jasco (Easton, MD, USA). A six-port LabPro valve (Rheodyne, Cotati, CA, USA) was used in the HPLC system to switch between mobile phases. ChromNAV v1.18.04 software and LCNet from Jasco were employed to control the chromatographic system and collect data. The chromatograms and peaks were analyzed by using PeakFit 4.12 (Jandel Scientific, Rafael, CA, USA). Nonlinear regression for the frontal analysis data was conducted by using DataFit 8.1.69 (Oakdale, PA, USA).

The analysis of AGP glycoforms by CE was accomplished by utilizing a P/ACE MDQ instrument (Beckman Instruments, Fullerton, CA, USA). The capillary was maintained at 25 °C and the applied potential was −30 kV. This separation used 60.2 cm × 50 μm I.D. fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) that had an effective length of 50 cm to the detector. The capillaries were modified by using both static and dynamic coatings of PEO [27]. Absorbance detection on the CE system was carried out at 200 nm.

2.3. Column preparation

Nucleosil 1000-7 silica was converted to a diol-bonded form as described previously [28]. This procedure involved first reacting the silica with 3-glycidoxypropyltrimethoxysilane in pH 5.5, 0.1 M sodium acetate buffer for 5 h at 90 °C. The epoxy groups on this support were then hydrolyzed to form diol groups by placing the modified silica in a pH 3.0 sulfuric acid solution and refluxing this slurry for 1 h [28]. A 3 mg portion of affinity-purified anti-AGP antibodies was immobilized onto 300 mg of this modified silica by using the Schiff base method, according to methods reported in Refs. [23,29]. A control support was prepared in the same manner but with no antibodies being added during the immobilization step (Note: No significant amount of binding was noted between the drugs examined in this study and the anti-AGP antibodies, but some drugs did show non-specific binding to the support, for which a correction was made by using this control material). The final supports were stored at 4 °C in pH 7.4, 0.067 M potassium phosphate buffer until use.