Effect of Endogenous Substances on the Binding of Sodium 4-Phenylbutyrate to Human Serum Albumin

Keishi Yamasaki¹, Koji Nishi², Taisuke Enokida¹, Kazuaki Taguchi¹ and Masaki Otagiri¹

¹Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto 860-0082, Japan
²Yokohama University of Pharmacy, Yokohama 245-0066, Japan

*Corresponding author: Masaki Otagiri, Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Nishi-ku, Kumamoto 860-0082, Japan, E-Mail: otagirim@ph.sojo-u.ac.jp

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Abstract

This study reports on the effect of endogenous substances such as fatty acids and indoxyl sulfate on the binding of sodium 4-phenylbutyrate (PB) to human serum albumin (HSA), using an ultrafiltration technique. The free concentration of PB in the filtrate was determined by HPLC. Fatty acids, particularly, octanoic acid are accompanied by a decrease in the binding of PB to HSA through an inhibitory displacement mechanism. The binding of indoxyl sulfate, a uremic toxin, to PB-HSA was decreased with increasing concentrations of caused of indoxyl sulfate. However, bilirubin showed a different behavior compared with octanoic acid and indoxyl sulfate: bilirubin had no effect on PB-HSA binding at lower concentrations of bilirubin, but the extent of binding was decreased at higher concentrations of bilirubin. The findings reported in this work provide basic and valuable information regarding our knowledge of altered PB binding that can occur in in certain diseased states.

Keywords: Human serum albumin; Sodium 4-phenylbutyrate; Endogenous substances; Displacement: Fatty acid; Indoxyl sulfate; Bilirubin.

Introduction

Human serum albumin (HSA) is the most abundant protein in the circulatory system, and one of its principal functions is the transport of endogenous substances such as fatty acids, hormones, toxic metabolites and bile acids [1]. HSA also binds a wide variety of drug molecules [2]. In the general circulation, drug molecules are either bound to plasma proteins or are present in an in an unbound (free) form. Certain unbound drug molecules, depending on their physico-chemical properties, can passively diffuse through physiological barriers. The binding of a drug to HSA only controls the free, active concentration of a drug. Thus, HSA-drug interactions are important factors in our understanding of the pharmacokinetics and pharmacological effects of various drugs.

In certain types of diseased states such as renal and hepatic diseases, the binding of drugs to HSA can be altered [3-6]. An understanding of the pharmacokinetics and pharmacological activity of drugs in a diseased state can provide important and useful information regarding the use of effective medication.

We recently reported that sodium 4-phenylbutyrate (PB), a drug that is used in treating urea cycle disorders, was found to bind to the site II area of HSA, based on binding experiments using chemically modified- and mutant-has preparations [7]. In our continuing investigations, the effects of endogenous substances, including fatty acids and uremic toxins, on the binding of
PB to HSA were examined with the objective of obtaining basic information regarding PB-HSA interactions in diseased states.

Materials and Methods

Materials

HSA (fraction V, fatty acid free) and bilirubin were purchased from Sigma Chemical Company (St. Louis, MO). PB was obtained from LKT Laboratories, Inc. (St. Paul, MN). Octanoic acid, oleic acid and myristic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Indoxyl sulfate was obtained from Nakalai Tesque (Kyoto, Japan). All other chemicals were purchased from commercial sources and were of the highest grade. Sodium phosphate buffer (pH, 7.4), at a concentration of 67 mM was used in protein-binding experiments.

Determination of the binding of PB to HSA in the absence and presence of endogenous substances

The binding of PB to HSA in the absence and presence of endogenous substances was determined using an ultrafiltration technique. Ultrafiltration was carried out using an AmiconR Ultra-0.5 mL centrifuge filter unit with an UltracelR-30 membrane (Merck Millipore Company, MA). Samples of 500 µL were centrifuged at 2,500 × g at 25 °C for 5 min. The concentration of unbound PB in the filtrates was then determined by HPLC.

Quantitative analysis of binding data

Data regarding albumin binding were quantitatively analyzed. Unbound (free) fractions were calculated using data obtained by ultrafiltration as follows.

\[
\text{Free fraction (\%) = } \frac{C_f}{C_f + C_b} \times 10
\]

Where \(C_f\) is the free PB concentration determined by measurement of the filtrate; \(C_b\) is the concentration of bound PB and was calculated by subtracting \(C_f\) from the total PB concentration (\(C_t\)).

Results

The concentration of serum albumin is altered in a variety of disease states including liver disease [3-6]. Such changes lower the extent of drug-HSA binding. It is also possible that the accumulation of endogenous substances such as fatty acids or uremic toxins could displace a drug that is bound to HSA.

To address this issue, we examined the effect of some representative endogenous substances including fatty acids and uremic toxins on the binding of PB to HSA. The presence of fatty acids, particularly octanoic acid, was found to lower the extent of binding of PB to HSA (FIG. 1).

The binding of indoxyl sulfate, a typical uremic toxin, induced a decrease in PB-HSA binding, which was decreased with increasing concentrations of indoxyl sulfate, as shown in FIG. 2. However, bilirubin behaved differently compared with octanoic acid and indoxyl sulfate. Bilirubin had no effect on the extent of PB-HSA binding at lower concentrations of bilirubin, but the binding to PB-HSA was decreased at higher concentrations of bilirubin (FIG. 3).

Discussion

Based on an X-ray crystallographic analysis of HSA, the molecule has the two specific drug binding sites, which are referred to as site I (warfarin site) and site II (diazepam site), as shown FIG. 4 [8-10]. Site I is made up of a pocket in subdomain IIA
and the inside wall of the pocket contains hydrophobic structures [11-13]. However, the entrance to the pocket is surrounded by positively charged residues. Site II is made up of a pocket in subdomain IIIA and is structurally similar to site I but site II appears to be somewhat smaller, or more narrow, compared with site I [11-13]. A number of factors can alter the binding of drugs to HSA. (2) One of these factors involves the displacement of a molecule that is bound to the binding site on HSA by endogenous substances, including fatty acids, that have accumulated in serum.

FIG. 1. Effect of Octanoic acid (open diamond), Oleic acid (open triangle) and Myristic acid (closed square) in PB binding to HSA at pH 7.4 and 25 °C. Each point represents the mean ± S.D. (n=3). *, P < 0.05, **, P < 0.01 in comparison with in the absence of ligands respectively.

FIG. 2. Effect of 3-indoxylsulfuric acid in PB binding to HSA at pH 7.4 and 25 °C. Each point represents the mean ± S.D. (n=3). **, P < 0.01 in comparison with the value in the absence of 3-indoxylsulfuric acid.
FIG. 3. Effect of bilirubin in PB binding to HSA at pH 7.4 and 25 °C. Each point represents the mean ± S.D. (n=3). **, P < 0.01 in comparison with the value in the absence of bilirubin.

FIG. 4. Overall structure of the HSA-PB complex [10]. HSA is shown as cartoon representations and the subdomain structures are colored in blue (IA), cyan (IB), pink (IIA), red (IIB), green (IIIA), and palegreen (IIIB). PB molecular is shown as CPK representations.

Octanoic acid, which strongly lowers PB-HSA binding, was used in this work. Other fatty acids are also known to inhibit PB-HSA binding. [13]. Whereas the inhibitory effects of the two fatty acids, namely, oleic acid and myristic acid, on the binding
of PB to HSA is much smaller (FIG. 1). Since octanoic acid binds to site II with a high affinity, a pronounced inhibitory effect of PB on the binding of site II ligands would be expected. The improving effect of long-chain fatty acids, such as myristic acid and oleic acid, was originally explained by the induction of conformational changes in the albumin molecule. [15, 18]. Indoxyl sulfate, uremic toxin, bind to site II, which is located in subdomain IIA of the HSA molecule. As expected, indoxyl sulfate reduced the binding of PB to HSA, but the inhibitory effect was clearly smaller than octanoic acid. The primary binding constants for the two ligands were nearly the same. Therefore, the difference in the inhibitory effects can be explained by differences in binding affinity (binding constant, K and the number of binding sites, n: nK). In fact, octanoic acid can bind to several sites [15, 16]. In addition, it is possible that conformational changes were induced. It should also be noted that the reduced drug binding observed in uremic patients is due to the direct and/or indirect displacement of the drug from the binding sites, which is caused by the increased levels of fatty acids and uremic toxins [19-24]. Bilirubin has the highest primary binding constant and binds to site I in subdomain IB, [25] suggesting that bilirubin exerts no inhibitory effects on the PB bound to HSA. However, the inhibitory effects on PB binding by bilirubin occurred at higher concentrations. This suggests that the bilirubin molecule, probably because of its rather large molecular size, overlaps partially the PB binding site. It is also possible that the binding of bilirubin to HSA may generate a change in the micro-environment of the PB binding site. The findings reported here provide basic and valuable information concerning our knowledge of alterations in PB binding in certain diseased states.

REFERENCES

22. Paolisso G, Tataranni PA, Foley JE. A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. Diabetologia, 38:1213-1217.