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Species Differences in the Binding of Sodium 4-Phenylbutyrate to Serum Albumin

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ABSTRACT

Sodium 4-phenylbutyrate (PB) is clinically used as a drug for treating urea cycle disorders. Recent research has shown that PB also has other pharmacologic activities, suggesting that it has the potential for use as a drug for treating other disorders. In the process of drug development, preclinical testing using experimental animals is necessary to verify the efficacy and safety of PB. Although the binding of PB to human albumin has been studied, our knowledge of its binding to albumin from the other animal species is extremely limited. To address this issue, we characterized the binding of PB to albumin from several species (human, bovine, rabbit, and rat). The results indicated that PB interacts with 1 high-affinity site of albumin from these species, which corresponds to site II of human albumin. The affinities of PB to human and bovine albumins were higher than those to rabbit and rat albumin, and that to rabbit albumin was the lowest. Binding and molecular docking studies using structurally related compounds of PB suggested that species differences in the affinity are attributed to differences in the structural feature of the PB-binding sites on albumins (e.g., charge distribution, hydrophobicity, shape, or size).

Introduction

Sodium 4-phenylbutyrate (PB) is a phenyl-substituted fatty acid derivative and is clinically used for the treatment of urea cycle disorders by virtue of its ammonium scavenging activity.[1,2] Recent research has focused on the action of PB as an inhibitor of endoplasmic reticulum (ER) stress[3,4] and histone deacetylases[5,6] and as a regulator of the hepatocanalicular transporter[7,8] in addition to its ammonium scavenging activity. Such a variety of actions of PB suggest that it has the potential for use in the treatment of a wider variety of diseases, in addition to urea cycle disorders. Indeed, in humans, it has been reported to have therapeutic effects on recurrent malignant gliomas and progressive familial intrahepatic cholestasis type 2.[2,9,10] In addition, several studies using cells and animal models suggest that PB has effects on neurodegenerative diseases,[11] inflammation,[12] cancers,[13] metabolic diseases,[3] hepatic fibrosis,[14] or spinal muscular atrophy.[15]

At the stage of development of PB as a drug with new indications, data from preclinical testing using experimental animals will be necessary to verify its efficacy and safety before it is approved for human use. Along with such pharmacodynamic data, pharmacokinetic data will be needed to understand or explain its pharmacodynamics and to determine the dosage requirements in humans. The binding of a drug to plasma proteins is one of the determinants of its pharmacokinetics (i.e., distribution, metabolism, and elimination).[16,17] Furthermore, the effective drug concentration at a pharmacologic target site is modulated by plasma protein binding because the effective drug concentration at a target site is proportional to the concentration of the unbound drug in the plasma.[18] Therefore, a knowledge of the binding of a drug to plasma proteins is an important factor in understanding or explaining the pharmacokinetics and pharmacodynamics of the drug.[18]

Abbreviations used: ER, endoplasmic reticulum; HDACs, histone deacetylases; M-PB, methyl 4-phenylbutyrate; PA, phenylacetic acid; PB, sodium 4-phenylbutyrate; PC, 6-phenylcaproic acid; PH, 7-phenylheptanoic acid; PP, 3-phenylpropionic acid; PV, 5-phenylvaleric acid.

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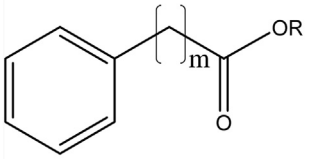

Compounds	m	R
PB	3	H[#]
PA	1	H
PP	2	H
PV	4	H
PC	5	H
PH	6	H
M-PB	3	CH_3

Figure 1. Chemical structures of PB and structurally related compounds (PA, PP, PV, PC, PH, and M-PB). [#]PB was used in the form of the sodium salt.

Albumin is a major protein component of blood plasma and plays an important role in the binding of many endogenous and exogenous compounds.[19] Human albumin contains 2 distinct ligand-binding sites, which are referred to as site I and site II.[20,21] X-ray crystallographic analyses indicate that human albumin contains 3 structurally similar α-helical domains, that is, I-III, which can be further divided into subdomains A and B, and site I and site II are located in subdomains IIA and IIIA, respectively.[22-24] The binding of a drug to the appropriate site on human albumin can be affected by the presence of other drugs or endogenous compounds or by a change in the microenvironment as the result of a diseased state.[17] Therefore, the identification and characterization of binding sites for certain drugs is also informative in terms of estimating the pharmacokinetics and pharmacodynamics of the drugs in several clinical situations. Mammalian albumin molecules have high sequence identities (72%-82%) and similarities (83%-88%) relative to human albumin,[25] and their domain structures are similar to those for human albumin,[26,27] suggesting that they contain the equivalent of sites I and II. Several studies done concerning the binding of drugs to albumins from several species have been reported.[28-35] Panjehshahin et al.[30] hypothesized that bovine, dog, horse, and sheep albumins contain binding sites that function similar to sites I and II of human albumin. However, they also implied that the binding sites for rat albumin are different from those of other albumins. Furthermore, we previously reported on the differences in structures of specific binding sites on human, bovine, dog, rabbit, and rat.[31] It has been reported that PB binds to a single site on human albumin, and the site was identified as site II.[36] The binding of PB to site II was suggested to be assisted by multiple forces such as hydrogen bonding, hydrophobic (and van der Waals), and electrostatic interactions, and that both Tyr411 and Arg410 play important roles in these interactions. However, considering the fact that animal models will be used to collect information on the protein binding of PB, our knowledge is very limited.

In the present study, we investigated species differences for the binding of PB to proteins. Albumin was initially identified as the main protein that binds PB in plasma of each of the species. We also compared the binding parameters for PB to mammalian albumin and those of structurally related compounds. Furthermore, computer modeling of the albumin-PB complex or complexes of structurally related compounds (Fig. 1) was performed to further evaluate the mechanisms that control the binding and structure of the PB-binding site at the molecular level, and the results for the various mammalian albumins were compared with each other.

Materials and Methods

Materials

Human, bovine, rabbit, and rat albumins, warfarin and dansylsarcosine, were purchased from Sigma Chemical Company (St. Louis, MO). Using the modified procedure reported by Chen,[37] the albumins were defatted with activated charcoal at 0°C in a solution acidified with H_2SO_4 to pH 3, deionized, and then freeze-dried. The resulting albumins gave only 1 band on SDS-PAGE, and their molecular masses were assumed to be 66 kDa when calculating molar concentrations. Sodium 4-PB was obtained from LKT Laboratories, Inc. (St. Paul, MN). Phenylacetic acid (PA) and 3-phenylpropionic acid (PP) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methyl 4-phenylbutyrate (M-PB) was obtained from Frinton Laboratories, Inc. (Hainesport, NJ). 5-Phenylvaleric acid (PV) and 6-phenylcaproic acid (PC) were purchased from Tokyo Chemical Industry Company Ltd. (Tokyo, Japan). 7-Phenylheptanoic acid (PH) was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX). Human and bovine plasma were purchased from the Interstate Blood Bank, Inc. (Memphis, TN) and DARD Company Ltd. (Tokyo, Japan). Whole blood was withdrawn from tail and auricular veins of anesthetized rabbits (white Japanese rabbit; male) and rats (Sprague Dawley rat; male), respectively. Rabbit and rat plasma were obtained by centrifuging whole blood samples. All other chemicals were purchased from commercial sources and were of the highest grade. About 67 mM of sodium phosphate buffer (pH, 7.4) was used in protein-binding experiments.

Determination of the Protein Binding of PB and Structurally Related Compounds to Mammalian Albumins

The binding of ligands to protein was determined by the method described in our previous report.[36] Briefly, ultrafiltration was carried out using Amicon® Ultra-0.5 mL centrifugal filter unit with an Ultracel®-30 membrane (Merck Millipore Company, Billerica, MA). Samples of 500 μL were centrifuged at 2500 *g* at 25°C for 5 min. Experiments were conducted at a temperature of 25°C so as to permit the data to be compared with previously reported data.[36] The filtrate represented about 5% of sample volume. The concentration of free (unbound) ligand in the filtrates (C_f) was quantified by HPLC system.[36] Bound fractions were calculated using the data obtained by ultrafiltration as follows.

$$\text{Bound fraction}(\%) = \frac{C_b}{C_b + C_f} \times 100\,, \tag{1}$$

where C_f is the free ligand concentration determined by measurement of the filtrate. C_b is the bound ligand concentration and was calculated by subtracting C_f from the total ligand concentration (before ultrafiltration) (C_t).

Binding parameters were determined by fitting the experimental data to the following equation using GraphPad PRISM®, version 7 (GraphPad Software, Inc., La Jolla, CA).

$$r = \frac{C_b}{P_t} = \sum_{i=1}^{m} \frac{n_i K_i C_f}{1 + K_i C_f}\,, \tag{2}$$

where r is the number of moles of ligand bound per mole of protein. P_t is the concentration of the protein. n_i is the number of binding sites, and K_i is the association constant in the ith binding class. In the case where the binding of PB to primary and secondary sites was characterized, the analysis was performed using $m = 2$. For the binding of structurally related compounds, the data under the condition, $r < 0.4$, to minimize ligand binding to any low-affinity binding sites, were applied in the analysis, and $m = 1$ was used in this case.

Fluorescent Probe Displacement

Fluorescent probe displacement experiments were performed using warfarin and dansylsarcosine, which are known to bind to