Contributions of Various Serum Proteins to the Binding of Prasterone Sulfate in Humans

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The binding of prasterone sulfate (PS) in human plasma was investigated. Binding percentages of PS to human plasma, human serum albumin (HSA), human α_1-acid glycoprotein (AGP) and human γ-globulin (GGL) were independent of the PS concentration between 0.1 and 8.0 μg/ml. The mean binding percentages were 99.1% for human plasma, 98.3% for HSA, 12.6% for AGP and 8.1% for GGL. Though PS is an acidic drug, binding of PS to AGP was observed. From the binding index, it was found that PS mainly bound to HSA in human plasma and that the contributions of AGP and GGL to PS in plasma were negligible.

Keywords prasterone sulfate; dehydroepiandrosterone sulfate; protein binding; human plasma; albumin; α_1-acid glycoprotein; γ-globulin

Introduction

Prasterone sulfate (PS) is an endogenous steroid secreted from the adrenals of primates,[1] and is generally known as dehydroepiandrosterone sulfate.

PS has a pharmacological effect on the insufficiency of uterine cervical maturation (insufficiency of orificial widening, and of cervical effacement and softening) in prelabor and is clinically used as an injectable preparation, Mylis® injection.

The binding of PS in human serum or plasma is more than 90%,[2-5] and binding to human serum albumin (HSA) is also high.[5] However, there is little information on the binding of PS to important serum proteins other than HSA, such as α_1-acid glycoprotein (AGP) and γ-globulin (GGL), and on the contribution of various serum proteins to the binding of PS.

It is generally assumed that acidic drugs are mainly bound to HSA in plasma.[6] However, the association constants of some acidic drugs to AGP are high enough to indicate that binding to AGP will contribute significantly to the total plasma binding.[6] On the other hand, there is a globulin to which steroids bind specifically, such as corticosteroid-binding globulin.[7]

The aim of this study was to estimate the relative contributions of various serum proteins to the binding of prasterone sulfate in humans.

Experimental

Drug and Chemicals The ^{14}C-labelled sodium PS and unlabelled sodium PS were identical to those used previously.[8] Commercial HSA, human AGP and human GGL were supplied by Sigma Chemical Co., (St Louis, MO, U.S.A.). All other chemicals used were commercially available and of analytical grade.

Plasma Human plasma samples were obtained from five healthy and drug-free female subjects (22 to 26 years old). The plasma of each subject was used for each experiment.

Equilibrium Dialysis Equilibrium dialysis was performed by the same way as described in detail previously.[8] A two-chambered apparatus of 2 ml capacity (Sanplatec Co., Osaka, Japan), separated by a cellulose dialysis membrane (Type 20/32, Sanko Junyaku Co., Tokyo, Japan) was used. HSA, AGP and GGL were dissolved at concentrations of 40, 0.9 and 1 mg/ml, in 1/15 M phosphate buffered isotonic saline, pH 7.4 (PBS). These concentrations were adjusted to the concentrations found in normal humans.[9] One ml of each protein solution containing PS was dialyzed against an equal volume of the PBS by agitation at 37 °C for 6 h. Total concentrations of PS were between 0.1 and 8.0 μg/ml. The binding experiment for plasma was carried out by the addition of unlabelled PS, and the concentrations of PS containing endogenous PS were measured by using a commercial radioimmunoassay kit (^{125}I-DHEA-Sulfate, ER-660, Baxter, Tokyo, Japan). The binding experiment for isolated serum protein was carried out by using ^{14}C-PS, and the radioactivity was measured according to the method described previously.[8]

Results

The binding percentages of PS to plasma, HSA, AAG and GGL at concentrations between 0.1 and 8.0 μg/ml are shown in Fig. 1. The bindings of PS to each protein were independent of the concentration of PS. The mean binding percentage was 99.1% for plasma, 98.3% for HSA, 12.6% for AGP and 8.1% for GGL.

Relative contributions of serum proteins to the binding of PS may be calculated as follows based on the binding index[10] under the condition of linear binding.

$$\text{binding index} = nP/kd = C_b/C_f = (C_t - C_f)/C_f$$

Where nP is the capacity of binding, kd is the dissociation constant, and C_b, C_f and C_t are the concentration of the bound drug, unbound drug (buffer solution side) and total drug (protein solution side), respectively. The calculated binding indexes for HSA, AGP and GGL were 56.8, 0.144 and 0.088 (100 : 0.25 : 0.15), respectively.

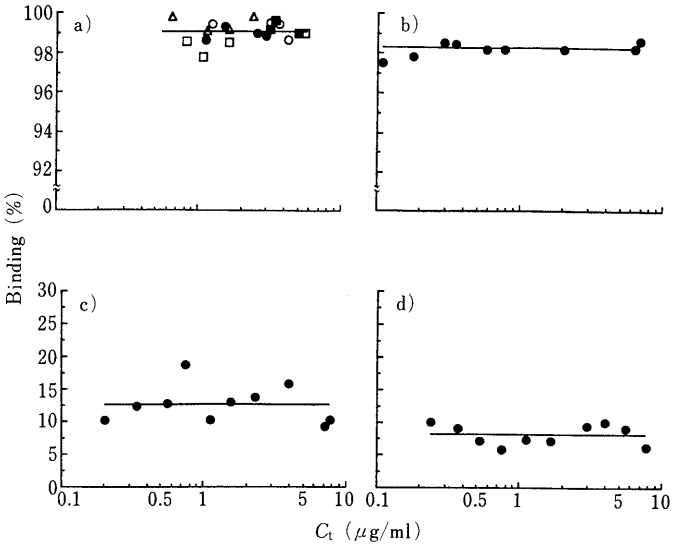

Fig. 1. Binding of PS to Human Plasma (a), HSA (b), AGP (c) and GGL (d)

Each symbol in panel (a) represents the data for each subject ($n=5$).

Discussion

Contributions of proteins in human plasma to the binding of PS were evaluated. The contributions of HSA, AGP and GGL to the binding of PS were 100 : 0.25 : 0.15, respectively, from the estimation of the binding index. Therefore, PS might bind mainly to HSA in human plasma, and contributions of AGP and GGL were negligible.

PS is an acidic drug with a sulfonic group. In general, acidic drugs are mainly bound to HSA.[6] However, the binding of some acidic drugs to AGP are so high that the binding to AGP is significant in the total plasma binding to these drugs.[6] Urien *et al.*[11] studied the plasma protein binding of several acidic drugs with or without a carboxylic group and found that clofibric, fenofibric, salicylic and valproic acids do not bind to AGP, and that benoxaprofen, indomethacin and itanoxone bind very poorly. In contrast, the percentage of bound warfarin, acenocoumarol and phenylbutazone are noticeably higher. The acidic drugs which exhibit a high or intermediate affinity to AGP do not have a carboxylic moiety. On the contrary, all the drugs exhibiting a poor affinity or no affinity to AGP have carboxylic groups. In this study, the binding of PS, which has a sulfonic group, to AGP was 12.5% (Fig. 1). Therefore, the binding of PS to AGP might be higher than that of acidic drugs with a carboxylic group.

Some steroids specifically bind to a certain globulin. For example, cortisol is bound to a specific transport protein, corticosteroid-binding globulin (CBG) with high affinity and low capacity which migrates on paper electrophoresis as an α_1-globulin.[7] But PS does not bind to CBG.[3] In this study, the binding of PS to γ-globulin (GGL) was examined. Consequently, the contribution of GGL to the PS binding in human plasma was found to be very small.

In conclusion, PS mainly binds to HSA in human plasma, and the binding of PS to AGP was also observed, though PS is an acidic drug with a sulfonic group.

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