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Short communication

Effect of the sialic acid residues upon the binding of beta blocker propranolol to human serum alpha-1 acid glycoprotein

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Introduction

In plasma, drugs are often bound to proteins such as albumin and alpha-1 acid glycoprotein (AGP). Plasma protein binding (PPB) of a drug has a significant effect on drug disposition and pharmacological activity (Meyer and Gultman, 1968; Vallner, 1977). One of the major physiological roles of AGP involves the binding and transportation of a range of endogenous and exogenous ligands. PPB is a focus of great importance in the pharmaceutical sciences as this interaction is a major factor in drug transport to tissue receptors, storage and prevention of metabolism (Kremer et al., 1988). In average healthy adults, the plasma concentration of AGP is approximately 10 – 20 μmol/L; whereas in diseased states, such as sepsis, it can increase up to 5-fold (Eap et al., 1991; Voulgari et al., 1982). Therefore, the effect of AGP binding on the pharmacological activity of highly bound drugs can be significant during acute phase reactions.

AGP is classified as one of the positive acute phase proteins (Kremer et al., 1988). It is composed of a single polypeptide chain which contains 183 amino acids with two disulfide bonds and five carbohydrate chains (Aubert and Loucheux-Lefebvre, 1976). The polypeptide component only contributes about a half of its total molecular mass of approximately 41 kDa, the rest of its mass derives from the five N-linked sialyl-glycans which confer AGP with a net negative charge at physiological pH. These features also render AGP very soluble and acidic (pI ~2.8 – 3.8) (Fournier et al., 2000; Schmid et al., 1977). The carbohydrate content of AGP is about 45 % (w/w), including 14 sialic acid residues per molecule (Schmid et al., 1977). The glycan structures show microheterogeneity under physiological conditions and the partially or fully desialylated AGP is known to exist in plasma of patients with liver disease (Serbource-Goguel Seta, 1983; Treuheit et al., 1992). Therefore, in certain disease states such as cancer, liver cirrhosis and inflammatory rheumatic disease, AGP has less sialic acid residues. In addition, it is expected that the sialic acid residues may be involved in different binding affinity and/or stereoselectivity because they contribute to the binding of some basic drugs to AGP such as propranolol.

The main goal of this study is to utilize isothermal titration calorimetry (ITC) to characterize the microscopic thermodynamic parameters that drive the binding of propranolol to native and desialylated AGP. However, the effect of sialic acid residues on binding by AGP is of interest both therapeutically and mechanistically not only for propranolol but also for other drugs administered orally that have a narrow therapeutic index, as this information could provide solutions for prescribing the correct drug dose in the clinic. In addition, changes in binding could lead to a change in the drug binding properties of AGP, which may have pharmacokinetic and pharmacodynamic implications.

Materials and methods

Materials

Human AGP (lot #018K7535) and (±)-propranolol were obtained from Sigma-Aldrich Chemie GmbH. All other reagents were of analytical grade or better.

Desialylation of human AGP

Desialylated AGP was prepared by incubation of immobilized sialidase beads (Immobilized SialEXO®) in the native human serum AGP buffered solution (2.5 mg/mL AGP, 25 mM HEPES, pH 7.4) at room temperature. After the incubation period of 24 hours, the desialylated sample was collected, washed out with water,

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and concentrated by centrifugal filtration (3 min 1 000 rcf).

ITC measurements

Microcalorimetric measurements of propranolol binding to native and desialylated AGP were performed on an MicroCal PEAQ ITC isothermal titration calorimeter (Malvern Panalytical Ltd, UK). The samples were thoroughly degassed beforehand. AGP samples (200 µM) in 25 mM HEPES buffer pH 7.4 were filled into the sample cell and titrated with a 1 mM propranolol solution in protein buffer dialysate at 250 s intervals. The cell contents were stirred constantly at 700 rpm. The ITC titrations were performed at 37 °C. Titrations for each different AGP sialylation form were repeated two or more times, using different working solution preparations as well as control experiments (drug into buffer) under the same injection conditions.

Results and discussion

Thermodynamic forces that drive the binding of propranolol to native (AGP+s) and desialylated (AGP-s), were characterized using modern ITC. The binding affinity (K_D) was determined to be 9.16 µM and 6.09 µM, respectively. Thus, the binding percent is 40.3 % which in turn indicates unsignificant binding. Thermodynamic signature for both bindings showed similar trend. Although, binding of propranolol to AGP-s displayed greater parameters. Binding enthalpy ($\Delta_r H°$), entropy ($\Delta_r S°$) and Gibbs free energy ($\Delta_r G°$) were 27.5 %, 77.3 % and 3.61 % different, respectively. Values were negative for both $\Delta_r H°$ and $\Delta_r G°$ which indicates an exothermic and spontaneous interaction and leads to favorable enthalpy while values for $\Delta_r S°$ were unfavorable. Negative $\Delta_r H°$ and $\Delta_r S°$ changes arise from hydrogen bond formation and van der Waals' interactions. High negative $\Delta_r H°$ values in the case of both bindings indicate that interaction is predominantly through hydrogen bonding interactions, especially since propranolol exists in ionized form at pH 7.4. Negative $\Delta_r S°$ value indicated a further contribution from van der Waal's interactions and more conformational changes.

Conclusion

ITC study suggests that the interaction of propranolol and AGP is an exothermic process and is driven mainly by enthalpy change. It indicated that the electrostatic interactions play a major role in the binding. The ITC study indicated approximately 1:1 stoichiometry. The removal of sialic acid dramatically diminished the conformational changes of AGP upon binding with propranolol, suggesting weaker interactions between AGP+s and propranolol. This study reported that ITC method could provide some valuable information to understand protein-drug interactions in drug development and optimization.

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