Sveučilište u Zagrebu

Farmaceutsko-biokemijski fakultet

Robert Kerep

UTJECAJ STUPNJA SIJALINIZACIJE LJUDSKOGA ALFA-1 KISELOGA GLIKOPROTEINA NA INTERAKCIJU S DILTIAZEMOM, IMATINIBOM, KLINDAMICINOM I LIDOKAINOM

DOKTORSKI RAD

Mentor:
Prof. dr. sc. Mario Gabričević

Zagreb, 2023.

University of Zagreb

Faculty of Pharmacy and Biochemistry

Robert Kerep

IMPACT OF THE SIALYLATION EXTENT OF HUMAN ALPHA-1 ACID GLYCOPROTEIN ON THE INTERACTION WITH DILTIAZEM, IMATINIB, CLINDAMYCIN AND LIDOCAINE

DOCTORAL DISSERTATION

Supervisor:
Professor Mario Gabričević, PhD

Zagreb, 2023

SAŽETAK

U današnje vrijeme sve se više istražuje vezanje liganda, poput lijekova i ksenobiotika, na proteine plazme. U ljudskome tijelu postoji više proteina plazme poput albumina, alfa-1 kiselog glikoproteina (AGP), lipoproteina i transferina koji su odgovorni za vezanje lijekova u cirkulaciji. Vezanje lijekova na proteine plazme važno je za sudbinu lijeka zbog utjecaja na njegovu farmakokinetiku i farmakodinamiku. AGP je protein akutne faze upale i njegova koncentracija u plazmi može se značajno povećati kod različitih bolesti ili trauma. Promjene u koncentraciji AGP-a mogu potencijalno utjecati na slobodni udio lijekova u plazmi. Nevezana frakcija ili slobodni udio lijeka je onaj za koji se smatra da je raspoloživ za aktivnost ili difuziju u okolna tkiva te se stoga samo slobodna frakcija lijeka smatra aktivnim oblikom, u skladu s tzv. „principom slobodnog lijeka“. Uslijed promjene koncentracije AGP-a, omjera njegovih genskih formi, kao i zbog njegovog visokog stupnja prirodne strukturalne varijabilnosti, posebice sijalinske kiseline, dolazi do promjene vezanja lijekova za AGP, ponajviše u patološkim stanjima. Vezanje lijekova za AGP može biti drugačije kao rezultat promjene u stupnju sijalinizacije. Promjene u glikozilacijskom obrascu sijalinske kiseline zamijećene su kod pacijenata s različitim vrstama karcinoma (primjerice, dojke, kolona, prostate), depresijom, infekcijama, insuficijencijom bubrega, itd. Navedeni primjeri jasno pokazuju važnost promjene strukture krajnjeg glikanskog dijela AGP-a što utječe na promijenjeno vezanje i distribuciju lijekova kod brojnih patofizioloških stanja.

U ovome radu koristila se izotermna titracijska kalorimetrija kako bi se odredile konstante ravnoteže reakcija vezanja i utjecaj stupnja sijalinizacije AGP-a prilikom vezanja s izabranim lijekovima te mikroskalarna termoforeza radi provjere interakcija AGP-a i lijekova. Glavni je cilj ovog rada kvantitativno procijeniti vezanje lijekova za nativni i desijalizirani AGP. Enzimska je desijalizacija AGP-a postignuta korištenjem imobiliziranog SialEXO® enzima. Dobiveni su kalorimetrijski podatci otkrili da je vezanje odabranih lijekova s AGP varijantama egzotermnog karaktera koje rezultira uglavnom entalpijski kontroliranim procesom, a konstanta ravnoteže disocijacije je u rasponu 1 – 32 μM. Desijalizirani AGP pokazao je različito vezanje s diltiazemom, imatinibom i lidokainom u usporedbi s nativnim AGP-om, dok s klindamicinom nije pokazao statistički značajnu razliku. Obzirom da sijalinska kiselina općenito uzrokuje različiti afinitet vezanja lijekova za AGP, klinički značaj desijaliziranog AGP-a ne bi se trebao zanemariti nego kao takav uzeti u obzir prilikom donošenja prikladne farmakoterapije.

Ključne riječi: alfa-1 kiseli glikoprotein, vezanje lijekova, konstanta ravnoteže reakcije vezanja, sijalinizacija, izotermna titracijska kalorimetrija

SUMMARY

Introduction

Nowadays, plasma protein binding is a focus of a great importance in pharmaceutical science and increasingly being studied. In the human body, there are several plasma proteins such as albumin, alpha-1 acid glycoprotein (AGP), lipoproteins and transferrin that are responsible for drug binding in the circulation. Protein-drug interaction is an important step for the fate of the drug due to its influence on pharmacokinetics and pharmacodynamics. AGP is an acute-phase protein and its plasma concentration can be significantly increased in various diseases. One of the major physiological roles of AGP involves the binding and transportation of a range of endogenous and exogenous ligands. Hence, changes in AGP concentration can potentially affect the free fraction of drugs in plasma. The unbound fraction or the free fraction of drug is the one that is considered available for activity or diffusion into the surrounding tissues, and therefore only the free fraction of the drug is considered as active form, in accordance with the „Free Drug Principle“. Due to the change in concentration of AGP, the ratio of its genetic forms as well as its high degree of natural structural variability, especially sialic acid residues, there is a change in the binding of drugs to AGP, mostly in pathological conditions. The binding of drugs to AGP may be different as a result of a change in the degree of sialylation. 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. 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. The above examples clearly show the importance of changing the structure of the terminal glycan part of AGP, which affects the altered binding and distribution of drugs in numerous pathophysiological conditions.

In this work, isothermal titration calorimetry has been used to determine the binding equilibrium constant and the influence of the degree of sialylation of AGP during the binding of selected drugs and consequently predict the impact of this change on therapy while microscale thermophoresis on the end to evaluate interactions. The main goal of this study is to define the microscopic thermodynamic factors that govern the binding of drugs to native and desialylated AGP using isothermal titration calorimetry. However, not only for examined drugs but also for other drugs administered orally that have a narrow therapeutic index, the effect of sialic acid residues on binding by AGP is of interest both therapeutically and mechanistically, as this

information could offer solutions for prescribing the proper drug dose in the clinic. AGP's drug-binding characteristics could also vary as a result of changes in binding, which could have an impact on its pharmacokinetic and pharmacodynamic features.

Materials and Methods

Human native AGP and examined drugs; clindamycin, diltiazem, imatinib and lidocaine were obtained from Sigma-Aldrich. All other reagents were of analytical grade or better.

The desialylated AGP was prepared through incubation of 500 μg of human native AGP buffered solution (25 mM phosphate buffer, pH 7.4) with immobilized sialidase beads (SialEXO) for 30 minutes at room temperature. The AGP sample was collected and concentrated using a 30 kDa molecular mass Amicon concentrator (Millipore, Billerica, MA). The concentration of the final AGP solution was measured by UV/Vis spectrophotometer (Cary 50, Varian). To confirm the characterization of desialylated AGP, samples were analyzed using UPLC-MS to ascertain complete desialylation of AGP. All glycan structures were annotated with MS/MS analysis using Synapt G2-Si ESI-QTOF-MS system (Waters, USA). Glycan compositions and structural features were assigned using software tools GlycoWorkbench and Glycomode, according to obtained MS and MS/MS spectra.

A MicroCal PEAQ-ITC calorimeter (Malvern, UK) was used for microcalorimetric experiments. AGP samples in 25 mM phosphate buffer pH 7.4 were filled into the sample cell and titrated with a 1→3 mM specific drug solution in AGP buffer dialysate at 250 s intervals. The cell contents were stirred constantly at 700 rpm. All of the ITC data were analyzed by using Analysis Software (Malvern) with a One Set Sites Fitting Model.

Microscale thermophoresis (Nanotemper, Germany) was also done by labelling AGP with Red-NHS dye using gel-filtration. After that, a serial dilution of drugs with labelled AGP was performed in order to monitor interaction by thermophoresis. Data were analyzed with MO. Affinity Software (Nanotemper).

Results

The affinity and the binding mode of AGP to examined drugs was evaluated by using ITC measurements, a powerful technique for estimation of all thermodynamic parameters in a single experiment, to reveal the changes in AGP-drug binding induced by the removal of terminal sialic acid. The binding of basic drugs from imatinib, diltiazem, lidocaine to clindamycin, all displayed an exothermically driven binding interaction with both native and desialylated AGP.

A correlation plot of the thermodynamic parameters for all examined drugs showed that an enthalpy–entropy compensation is in effect. The exothermic binding of the drugs were driven by a combination of favorable (negative) enthalpic ($\Delta_r H°$) and favorable (positive) entropic ($\Delta_r S°$) contributions to the Gibbs free energy ($\Delta_r G°$). This may indicate that the binding actually happened. A negative value of $\Delta_r H°$ is typically interpreted as proof of electrostatic interaction and hydrogen bonds. It is thought that the positive value of change in entropy is a sign of hydrophobic interactions and adds contribution to the negative Gibbs free energy. The equilibrium dissociation constant was in the range between 1 and 32 μM. Desialylated AGP showed approximately 42 %, 45 % and 62 % different binding affinity with diltiazem, lidocaine and imatinib, respectively, compared to native AGP, while clindamycin showed no statistically significant difference (~6 %). Considering that imatinib and diltiazem showed notable difference for the AGP sialo-forms resulting in a decrease of the free fraction in blood at peak levels by about 32 % and 25 %, respectively, compared to the native (low plasma concentrations) AGP, such an observation is of crucial importance for further pharmacokinetic studies.

Conclusion

This study reported that isothermal titration calorimetry could provide some valuable information to understand protein-drug interactions. Isothermal titration calorimetry suggests that the interaction of drugs with AGP sialoforms were an exothermic process and is driven mainly by enthalpy. The calorimetric measurements and statistical approach indicated that there was significant difference in binding of diltiazem, lidocaine and imatinib between native and desialylated AGP. The drastic reduction in conformational changes caused by the elimination of sialic acid residues from AGP suggests stronger interactions between desialylated AGP and abovementioned drugs. The results discussed here may be relevant information for thinking towards safer and more effective drug use treatments.

Keywords: alpha-1 acid glycoprotein, drug binding, equilibrium binding constant, sialylation, isothermal titration calorimetry