Pharmacological Reports 66 (2014) 727–731

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Short communication

Phenylbutazone and ketoprofen binding to serum albumin. Fluorescence study

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ARTICLE INFO

Article history:
Received 16 February 2014
Received in revised form 18 March 2014
Accepted 18 March 2014
Available online 3 April 2014

Keywords:
Competition
Spectrofluorescence
Serum albumin

ABSTRACT

Background: A combination of phenylbutazone (PBZ) and ketoprofen (KP) is popular in therapy of rheumatoid arthritis (RA) but could be unsafe due to the uncontrolled growth of toxicity.
Methods: Quenching fluorescence of serum albumin in the presence of the both drugs has been characterized by dynamic K_Q [M^{-1}], static V [M^{-1}] quenching constants and also association constants K_a [M^{-1}].
Results: The quenching of tryptophanyl residues fluorescence by the KP and PBZ indicates the capability of these drugs to accept the energy from Trp-214 and Trp-135. Strong displacement of KP and PBZ bound to albumin cause by the binding of the second drug to SA close to Trp-214 (subdomain IIA) has been obtained. The displacement was also confirmed on the basis of quenching and association constants.
Conclusions: The conclusion, that both PBZ and KP form a binding site in the same subdomains (IIA or/and IB), points to the necessity of using a monitoring therapy owning to the possible increase of the uncontrolled toxic effects.

Introduction

Rheumatoid arthritis (RA) results from inflammation of the joints that have been attacked because of a faulty immune system. Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to reduce pain as well as inflammation in the joints. When two drugs are administered together the interaction and competition between them for the binding site on albumin may lead to the increase of unbound drug fraction in blood and evoke adverse/toxic effects. Phenylbutazone (PBZ) and ketoprofen (KP) are commonly used together in combination therapy of RA. Phenylbutazone and ketoprofen bind to plasma proteins, primarily to albumin, by 98–99%. Serum albumin (SA) is the most abundant plasma protein. The binding of drugs to plasma protein and thus formation of drug plasma protein complex is an important factor affecting their distribution and rate of metabolism. The binding mainly takes place in subdomains IIA and IIIA, known as Sudlow's sites I and II. The interaction of drug with serum albumin is important component in understanding the mechanism of action, especially, drug distribution and interaction with SA. Binding of one drug molecule to SA often influences simultaneous binding of another drug [1]. It can lead to the displacement of one drug by another in the binding site [2]. NSAIDs have high protein binding that may represent displacement of bound drugs especially those bound to albumin. Many studies have confirmed a competition between drugs in binding to the primary binding site on albumin [1–3]. Because both PBZ and KP show a high affinity for the serum albumin binding site, the possibility of displacement from secondary binding site to another site seems to be important to study and should be taken into account.

Materials and methods

Reagents

Bovine serum albumin (BSA) and ketoprofen (KP) were purchased from MP Biomedicals (USA), human serum albumin (HSA) from ICN Biomedicals (USA). Phenylbutazone (PBZ) was provided by Sigma–Aldrich Chemical Co. (Germany). Sodium dihydrogen phosphate dehydrate ($Na_2HPO_4{\cdot}2H_2O$), dipotassium phosphate trihydrate ($K_2HPO_4{\cdot}3H_2O$) and methanol were purchased from POCH (Poland).

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http://dx.doi.org/10.1016/j.pharep.2014.03.005
1734-1140/

Fluorescence quenching measurements

Emission fluorescence spectra were recorded on a Kontron Instrument AG spectrofluorimeter. To excite the SA fluorophores 295 nm wavelength was used. All solutions were prepared using 0.05 M sodium phosphate buffer (pH 7.4 ± 0.1) and incubated overnight. Ketoprofen and phenylbutazone solutions were initially dissolved in methanol (not exceeding 1% v/v in the final concentration).

To obtain the binary complexes (KP-SA, PBZ-SA) the solution of SA was titrated by ketoprofen and phenylbutazone solution. To obtain the ternary complexes (KP-[PBZ]-SA, PBZ-[KP]-SA) the solution of SA and appropriate volumes of first solution (to get molar ratio drug-albumin 1:1) was titrated by a second ligand.

The intensity of fluorescence was corrected for the inner filter effect [4]. The UV–VIS absorption does not exceed 0.3 near excitation and emission wavelength. Therefore the correction for fluorescence intensity by the above equation was credible [5].

Dynamic K_Q [M^{-1}] and static V [M^{-1}] quenching constants were calculated according to the modified Stern–Volmer nonlinear regression equation [6]. The association constants K_a [M^{-1}] were estimated using the Scatchard method [7].

To calculate the percentage of displacement of KP (PBZ) from its binding site in albumin by PBZ (KP) the relationship was used:

$$\frac{F_0 - F}{F_0} \times 100\% \quad (1)$$

where F_0 and F are the fluorescence intensities of KP (PBZ) – SA in the absence and presence of ligands.

Results and discussion

Effect of phenylbutazone and ketoprofen on serum albumin fluorescence. Comparative study

Ketoprofen (KP), an arylpropionic acid that contains a benzophenone moiety, is used as a photolabel of the binding region of Sudlow's site II of serum albumin (SA) in subdomain IIIA [8]. For ketoprofen binding to SA the most important are the guanidine moiety of Arg-410, the phenolic oxygen and the aromatic ring of Tyr-411, which is, protrude toward the center of site II [9]. The guanidine moiety probably interacts electrostatically with the carboxyl group of ketoprofen, the phenolic oxygen could make a hydrogen bond with the keto group of the ligand, and the aromatic ring may participate in a specific stacking interaction (π–π) with one or both of the aromatic rings of ketoprofen [9]. A wavelength of 295 nm used in the study excites only tryptophanyl residues, i.e. Trp 135, 214 located in subdomains IB and IIA of BSA and Trp 214 in subdomain IIA of HSA. Fluorescence quenching of SA can be used to obtain many ligand-albumin binding information. When the fluorescence emission spectra of the donor and the absorption spectra of the acceptor have enough overlap and the distance between them should not exceed 10 nm, the energy transfer can occur [10]. Then, the albumin fluorescence could be quenched. The decrease of SA fluorescence intensity was observed at emission maxima for KP-SA (Fig. 1) and PBZ-SA (data not shown) systems. Addition of increasing amounts of ketoprofen and phenylbutazone causes the fluorescence of SA quenched. At KP:SA and PBZ-SA molar ratios 6.25:1 quenching fluorescence equals to almost 60–70% and was higher than that caused by PBZ. When absorbance at excitation and emission fluorescence was within 0.05 and 0.3 correction for the inner filter effect (IFE) was done (KP-SA system). Absorbance of PBZ at the concentration used was $\leq$0.05 and the fluorescence spectra have not been corrected. The quenching of tryptophanyl residues fluorescence by KP and PBZ indicates the capability of KP and PBZ to accept the energy from

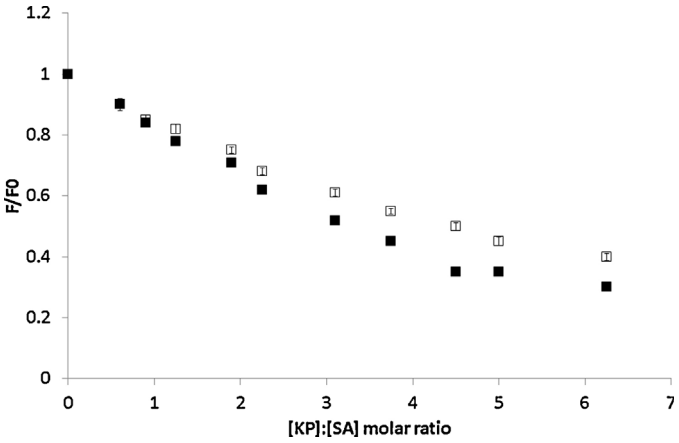

Fig. 1. Quenching curve of bovine (■) and human (□) serum albumin (8×10^{-6} [mol/L]) in the presence of KP (5×10^{-6} [mol/L]–5×10^{-5} [mol/L]), [KP]:[SA] 0.625:1–6.25:1; λ_{ex} = 295 nm; T = 310 K. Error bars represent standard deviation.

Trp-214, 135, i.e. two excited fluorophores, and thus location in human and bovine serum albumin subdomain IIA and IB, respectively. The aromatic residues of albumin form by specific stacking interaction with one or both of the aromatic rings of ketoprofen and phenylbutazone a sandwich-type complex. The quenching curves of HSA excited at λ_{ex} = 295 nm in the presence of KP (Fig. 1) have been compared with those of BSA. The quenching curves of SA do not overlap. Fluorescence quenching of BSA by KP is more extended when compared to HSA. This effect points to alterations within Trp 214 and 135 microenvironments caused by the presence of KP means that besides of the primary binding site in subdomain IIIA the secondary KP-SA binding site in subdomain IIA or IB may exist. The π–π interaction between tryptophanyl residues (Trp-214, 135) and the aromatic ring of ketoprofen has been suggested basing on results presented in Fig. 1. With the increase of KP:SA molar ratio from 0.625:1 to 6.25:1 the decrease in both albumin fluorescence has been observed. The capability of phenylbutazone to accept the energy from the excited fluorophores of SA was observed by many scientists. Sułkowska et al. [1] observed that primary high affinity binding site and secondary binding site in serum albumin for PBZ are placed in subdomain IIA where Trp-214, 263 are located and IIIA of serum albumin. These sites are characteristic for a hydrophobic interaction. PBZ forms a "sandwich structure" with aromatic residues of SA and subdomain IIA is a major binding site for PBZ [11].

The quenching curves for PBZ-HSA and PBZ-BSA systems did not overlap. This indicates that Trp-135 located in the subdomain IB participates in the formation of PBZ-BSA complex. The results obtained show that tryptophanyl residues of albumin are accessible to KP and PBZ. Both, KP and PBZ form "sandwich-type" complex in the IIA subdomain, between their aromatic rings and the aromatic residues of tryptophan(s). The analysis of the simultaneous binding of these two drugs in Sudlow's site I as well as possible competition between them in the binding within subdomain IIA (primary binding site for phenylbutazone and secondary binding site for ketoprofen) seemed to be necessary.

Fluorescence intensity data in KP-SA (Fig. 2) and PBZ-SA (data not shown) systems were analyzed according to Stern–Volmer dependence [12]. The dependence of F_0/F on concentration of KP (Fig. 2) when only tryptophanyl residues have been excited at 295 nm allows for description of both collisional (dynamic) and static quenching in subdomain IIA or IB. Linear dependence points to dynamic quenching. The Stern–Volmer plot showed a linear relationship between fluorescence intensity and quencher concentration [KP] below 2×10^{-5} [mol/L] in KP-SA (Fig. 2) and