Colloids and Surfaces B: Biointerfaces 145 (2016) 820–829

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Studies on the interaction between promethazine and human serum albumin in the presence of flavonoids by spectroscopic and molecular modeling techniques

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ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 31 May 2016
Accepted 1 June 2016
Available online 2 June 2016

Keywords:
Flavonoids
Promethazine
Human serum albumin
Competitive binding
Food-drug interaction

ABSTRACT

Fluorescence, absorption, time-correlated single photon counting (TCSPC), and circular dichroism (CD) spectroscopic techniques as well as molecular modeling methods were used to study the binding characterization of promethazine (PMT) to human serum albumin (HSA) and the influence of flavonoids, rutin and baicalin, on their affinity. The results indicated that the fluorescence quenching mechanism of HSA by PMT is a static quenching due to the formation of complex. The reaction was spontaneous and mainly mediated by hydrogen bonds and hydrophobic interactions. The binding distance between the tryptophan residue of HSA and PMT is less than 8 nm, which indicated that the energy transfer from the tryptophan residue of HSA to PMT occurred. The binding site of PMT on HSA was located in sites I and the presence of PMT can cause the conformational changes of HSA. There was the competitive binding to HSA between PMT and flavonoids because of the overlap of binding sites in HSA. The flavonoids could decrease the association constant and increase the binding distance. In addition, their synergistic effect can further change the conformation of HSA. The decrease in the affinities of PMT binding to HSA in the presence of flavonoids may lead to the increase of free drug in blood, which would affect the transportation or disposition of drug and evoke an adverse or toxic effect. Hence, rationalising dosage and diet regimens should be taken into account in clinical application of PMT.

1. Introduction

Drug-drug interaction, including pharmacodynamics and pharmacokinetic interaction, is defined as an interaction between a drug and another substance that prevents the drug from performing as expected. Meanwhile, the interaction may also exist between drugs and foods (food-drug interaction), as well as drugs and herbs (herb-drug interaction). This action can be synergistic, antagonistic or a new effect neither produced by its own [1,2]. A drug's efficiency is affected by the degree of its bind to plasma proteins because the most drugs are transported as complexes combined with plasma proteins, and only free drugs can cross the biological membrane to produce pharmacological effects [3,4]. Usually, co-administration of two drugs will change the affinity of plasma proteins towards a drug because of the overlap of binding sites or conformational changes of proteins [5–7]. The competitive bind to plasma proteins between two drugs belongs to pharmacokinetic interactions, which will result in the change of the concentration of the free biologically active fraction of one or two drugs, and then influence the therapeutic effect, side effect and toxicity of drugs [8,9]. Therefore, the investigations of drug-plasma proteins interactions in the presence of other endogenous or exogenous compounds play an important role for the interpretation of drug–drug interactions [9,10].

Promethazine hydrochloride (PMT) is chemically named as (2RS)-*N*,*N*-dimethyl-1-(10H-phenothiazin-10-yl) propan-2-amine hydrochloride (Table 1). It is one of the most important compounds of the phenothiazine derivatives and structurally different from the neuroleptic phenothiazines, with similar but different effects [11]. It acts primarily as a strong antagonist of the H_1 receptor (antihistamine) and a moderate mACh receptor antagonist (anticholinergic) [11,12], and also has weak to moderate affinity for the 5-HT_{2A}, 5-HT_{2C}, D_2, and α_1-adrenergic receptors [13,14], where it

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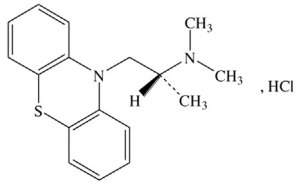
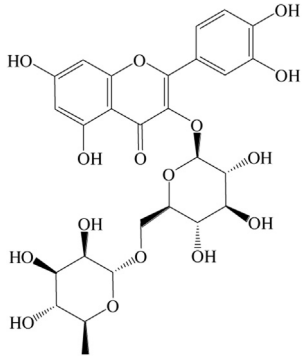
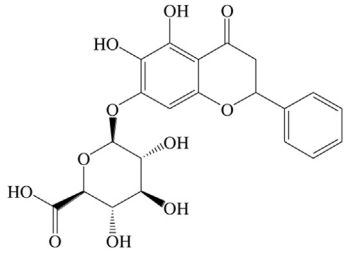
http://dx.doi.org/10.1016/j.colsurfb.2016.06.001
0927-7765/

Table 1
Structural formula of PMT, rutin and baicalin.

Name	Molecular formula	Structural formula
PMT	$C_{17}H_{20}N_2S{\cdot}HCl$	
Rutin	$C_{27}H_{30}O_{16}$	
Baicalin	$C_{21}H_{18}O_{11}$	

acts as an antagonist, as well. Another notable use of PMT is as a local anesthetic by blockade of sodium channels [14]. Its common side effects include: tardive dyskinesia, confusion in the elderly, drowsiness, dry mouth, respiratory depression, constipation, chest discomfort, and so on. The interaction between PMT and other drugs should be taken into account in clinical application of PMT because the drug–drug interaction may increase the risk of side effects from occurring. For example, patients taking PMT with ketorolac can cause delirium [15]. The character of kinetic curves of promethazine changed substantially and the elimination of PMT in the blood was prolonged under the influence of carbamazepine and chlorpromazine [16,17]. In addition, patients should be warned not consume anything (drugs and foods) containing caffeine at the treatment by PMT, as caffeine will cause increasing of maximum and current concentration of PMT and its prolongation in organism [18], which indicates that the food-drug interaction should be considered in the period of the drugs treatment.

Flavonoids are a class of important polyphenols compounds with diphenylpropane ($C_6C_3C_6$) skeleton found in nature. They are widely distributed in human diets such as fruits, vegetables, wine, seeds or roots of plants [19,20]. Up to now, more than 7000 varieties of flavonoids have been identified and can be classified as flavones, flavonols, flavanones, isoflavones, flavan-3-ols and anthocyanidins according to their structural difference [21,22]. Flavonols and flavones among them are two kinds of flavonoids most widely distributed in various plants [23]. Usually, flavonoids in plants are combined with one or more sugar molecules and known as flavonoid glycosides [24]. Accumulating evidence suggests that flavonoids exhibit extensive pharmacological effects and biological effects in vitro and in vivo including antioxidant, anti-inflammation, anti-microbial, anti-tumor, anti-asthma, anti-adipogenesis and anti-diarrhea [19,20,23–26]. Because of the potential beneficial effects of flavonoids on human health, foods rich in flavonoids have attracted great interest recently [25,26]. It has been reported that the polyphenols of average human dietary intake is about 1 g/day, with two-third being flavonoids [27]. In addition, the current reports show that the flavonoids can competitive bind with many drugs and affect their binding affinity to the plasma proteins [10,28–30]. However, to the best of our knowledge, the competitive binding between flavonoids and PMT to the plasma proteins has not been reported.

Rutin belongs to flavonols and is a glycoside between the quercetin and the disaccharide rutinose (α-L-rhamnopyranosyl-(1 → 6))-β-D-glucopyranose). Baicalin belongs to flavones and is the glucuronide of baicalein (Table 1). In this work, the two flavonoids were selected as the representatives of flavonols and flavones used to study their influence on the binding affinity of PMT to the plasma proteins. Common plasma proteins binding with drugs are human serum albumin (HSA), lipoprotein, glycoprotein, and α, β‘ and γ globulins [31]. HSA is the most abundant protein in plasma. It is frequently used in biophysical, biochemical and physicochemical studies because of a well-known primary structure [32–34]. Herein, HSA was selected as the model of plasma proteins. The binding properties of PMT to plasma proteins in vitro in the presence and absence of two kinds of flavonoids, rutin and baicalin, were investigated by fluorescence, absorption and circular dichroism (CD) spectroscopy and molecular modeling method. We hope that this study can provide important insight into the clinical research and the theoretical basis for pharmacokinetics.

2. Materials and methods

2.1. Materials

HSA (Fraction V, purity 96–99%) was obtained from Beijing Solarbio Science & Technology Co., Ltd. (China). The HSA stock solution, 1.0×10^{-5} mol/L, was prepared in 0.05 mol/L Tris-HCl buffer solution of pH 7.40 containing 0.05 mol/L NaCl, then stored at 4 °C in the refrigerator. PMT was obtained from the Liaoning Donggang Hongda Pharmaceutical Co., Ltd. (China). Ibuprofen was obtained from Sun Chemical Technology (Shanghai) Co., Ltd. (China). Warfarin was obtained from Dalian Meilun Biotech Co., Ltd. (China). Rutin and baicalin were obtained from Chengdu Superman Plant & Chemical Development Co., Ltd. (China). The stock solutions of PMT, ibuprofen, warfarin, rutin and baicalin were prepared by dissolving them in a small amount of ethanol, and then diluting them to 2.51×10^{-3} mol/L with the same buffer solution above. All other reagents were commercial products of analytical grade.

2.2. Apparatus

The fluorescence emission spectra and synchronous fluorescence spectra were carried out on an F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Japan). The absorption spectra were recorded on a UV-2550 spectrophotometer (Shimadzu Co., Japan). The specified temperatures were controlled by an SC-15 digital aqueous thermostat (Shanghai Bilon Instrument Co., Ltd., China). The CD spectra were recorded on a J-810 spectropolarimeter (Jasco) equipped with a 1.0 cm path length quartz curette under nitrogen protection. The time-resolved fluorescence spectra were collected using a DeltaFlex time-correlated single photon counting (TCSPC) spectrometer (HORIBA Jobin Yvon IBH Ltd., U.K.).