Journal of Photochemistry & Photobiology, B: Biology 175 (2017) 192–199

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Studies on the competitive binding of cleviprex and flavonoids to plasma protein by multi-spectroscopic methods: A prediction of food-drug interaction

Xin Wang[a,b], Xue-Yuan Guo[b], Liang Xu[a,b], Bin Liu[b], Ling-Ling Zhou[b], Xiao-Fang Wang[b], Dan Wang[b], Ting Sun[a,*]

[a] Department of Chemistry, College of Science, Northeastern University, Shenyang 110819, China
[b] School of Pharmaceutical Sciences, Liaoning University, Shenyang 110036, China

ARTICLE INFO

Keywords:
Flavonoids
Cleviprex
Food-drug interaction
Competitive binding
Plasma protein

ABSTRACT

Cleviprex is a short-acting dihydropyridine calcium channel antagonist used as an antihypertensive drug. In this work, the binding characterization of cleviprex to human serum albumin (HSA) and the competitive binding to HSA between cleviprex and two flavonoids, baicalin and rutin, were studied using multi-spectroscopic techniques and molecular docking method. The fluorescence quenching of HSA by cleviprex was initiated by the formation of HSA-cleviprex complex, which was confirmed by UV–vis spectra measurements. The results of thermodynamic analysis and molecular docking revealed that the hydrophobic interactions and hydrogen bonding were the major acting forces in stabilizing HSA-cleviprex complex. The results of substitution experiments and molecular docking demonstrated that cleviprex was mainly situated within the site I of HSA. Baicalin and rutin could reduce the values of binding constant and enhance the values of binding distance of cleviprex binding to HSA because they bind to the same binding site. The results of synchronous fluorescence and CD spectra suggested that the binding reaction of cleviprex to HSA could give rise to the changes of protein conformation and the combined actions of cleviprex and flavonoids could cause further changes of HSA conformation. Consequently, the intakes of flavonoid-rich foods and beverages should be lessened under the treatment of cleviprex to avoid food-drug interactions.

1. Introduction

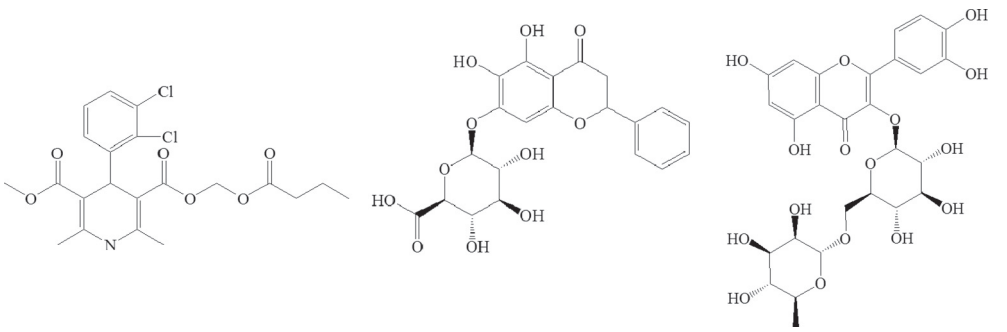
Flavonoids are a large class of naturally occurring bioactive polyphenols widely present in plant foods and beverages [1,2]. Until now, over 6000 flavonoids have been found [3]. According to their structural difference, they are mainly categorized into flavones, flavonols, anthocyanidins, flavanones, isoflavones and flavan-3-ols [4–7]. Numerous investigations have described that flavonoids have many beneficial pharmacological activities, including cardiovascular effects, antioxidant, anti-inflammatory, antiallergic, antimicrobial, antithrombotic, antiviral, antidiabetic, estrogenic and anticarcinogenic activities [3,8,9]. Because they are very abundant in our diets and many studies have suggested that modest long-term ingestions of flavonoids have health-promoting effects, flavonoid-rich foods and beverages have attracted great interest recently [1,10,11].

Food-drug interaction is recognized that the simultaneous intakes of food and drug cause changes in pharmaceutical, pharmacokinetic, or pharmacodynamic properties of foods or drugs. Some food-drug interactions may be benefit to the patients, but mostly they may induce adverse drug reactions. Accordingly, patients should follow the instructions of doctors on the proper use of drugs to avoid serious side effects [12,13]. Most drugs bind reversibly to the plasma proteins and are transported in the circulatory system [14]. Consequently, the affinity of drug binding to plasma proteins affects the magnitude of its biological actions in vivo [14,15]. The fraction unbound of a drug can be altered by other ligands that binding to plasma proteins. Accordingly, the competitive binding to plasma proteins between drugs and food components is a reason of the generation of food-drug interaction [16,17]. Therefore, the investigations on the competitive binding to plasma proteins between drugs and food components play an important role in pharmacology and pharmacokinetics [18,19].

Cleviprex (the structure shown in Fig. 1) is a short-acting dihydropyridine calcium channel antagonist used as an antihypertensive drug [20]. Clinical trials have demonstrated that it can be used to effectively manage the acute hypertension for patients [20,21]. It has been reported that grapefruit juice can change the area under the curve

* Corresponding author.
E-mail address: sun1th@163.com (T. Sun).

http://dx.doi.org/10.1016/j.jphotobiol.2017.08.037
Received 17 April 2017; Received in revised form 12 July 2017; Accepted 27 August 2017
Available online 04 September 2017
1011-1344/



Cleviprex Baicalin Rutin

Fig. 1. Molecular structure of cleviprex, baicalin and rutin.

ratio of cleviprex [22], which is indicative of further studies of food-cleviprex interactions are needed. The binding of cleviprex to plasma proteins is more than 99.5% [23], which indicates that the factors can alter the affinity of cleviprex binding to plasma proteins should be considered in clinic. In addition, it has been reported that the flavonoids intake from the foods and beverages of people is concerning 0.67 g/day [10]. Many reports suggest that flavonoids can affect the binding characterization of many drugs to plasma proteins [4,24–26]. Therefore, investigation on the influences of food components, such as flavonoids, on the binding of cleviprex to plasma proteins is of fundamental importance in clinic. However, the flavonoids-cleviprex interactions such as the competitive binding to plasma proteins between cleviprex and flavonoids are still insufficient report to our knowledge.

Human serum albumin (HSA) is the most abundant plasma protein and it is about 60% of total protein in blood [27]. Its molecular structure enables it to reversibly bind various endogenous and exogenous compounds such as fatty acids, drugs and food components [28]. Because it is responsible for the majority of ligand binding to plasma proteins and its well-known primary structure, HSA is usually used as a model of plasma proteins to study the binding properties of various endogenous and exogenous compounds bind to plasma proteins [27,29–32]. Herein, the binding properties of cleviprex to HSA and its competitive binding to HSA with two kinds of the most widespread flavonoids, flavones (e.g. baicalin, the structure shown in Fig. 1) and flavonols (e.g. rutin, the structure shown in Fig. 1), were investigated by multi-spectroscopic techniques and molecular docking method. It is wished that the study can offer beneficial information for studying the pharmacokinetics of cleviprex and its food-drug interaction.

2. Methods and Materials

2.1. Chemicals and Reagents

Cleviprex (purity, 98%) was purchased from Shanghai Civi Chemical Technology Co., Ltd. China. Baicalin (purity, 99%) was purchased from Hunan Province Waynick Biological Technology Co., Ltd. China. Rutin (purity, 98%) was purchased from Xi'an Saiyang Bio-Technology Co., Ltd. China. Ibuprofen (purity, 99.5%) was purchased from Zhengzhou Debao Fine Chemical Co., Ltd. China. Warfarin (purity, 98%) was purchased from Tianjin Elong Co., Ltd. China. HSA (Fraction V, purity, 96–99%) was purchased from Beijing Solarbio Science & Technology Co., Ltd. China.

2.2. Fluorescence Spectra Measurements

All fluorescence spectra were measured on a fluorescence spectrophotometer (Model F-7000, Hitachi High-Technologies Co., Japan) equipped with a 150 W xenon lamp, a 1.0 cm quartz cuvette and a thermostat bath (Model SC-15, Shanghai Bilon Instrument Co., Ltd., China). The HSA solution was prepared by the 0.05 mol/L NaCl-Tris-HCl buffer solution (pH 7.40). The cleviprex, flavonoids and specific binding site markers were dissolved in 2 mL ethanol, and then attenuated to the certain concentration by the 0.05 mol/L NaCl-Tris-HCl buffer solution (pH 7.40). In the experiment, the different volume of stock solutions were mixed together and the final concentration of HSA was 1.00×10^{-5} mol/L. The fluorescence emission spectra at 288, 301 and 310 K were measured from 290 to 490 nm at an excitation wavelength of 280 nm. The excitation and emission band widths were both 5.0 nm while the scanning rate was 1200 nm/min and the photo multiplier tube voltage was 700 V. In order to subtract the inner-filter effect, the fluorescence intensity data used in this paper were corrected by the following equation [33,34]:

$$F_{cor} = F_{obs} \times e^{\frac{A_{ex}+A_{em}}{2}} \quad (1)$$

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensities data, respectively. A_{ex} and A_{em} are the absorbance of protein-ligand mixed solutions at the excitation and emission wavelength, respectively. The synchronous fluorescence spectra were measured by simultaneously scanning the excitation and emission monochromators when the wavelength interval was 15 or 60 nm.

2.3. UV–vis Spectra Measurements

The UV–vis spectra were measured from 200 to 800 nm on a spectrophotometer (Model UV-2550, Shimadzu Co., Japan) at 288 K. The concentration of HSA was 0.0 or 1.0×10^{-5} mol/L and the concentrations of cleviprex changed from 0.0 to 8.0×10^{-5} mol/L at 1.0×10^{-5} mol/L intervals.

2.4. CD Spectra Measurements

The CD spectra were measured from 200 to 250 nm on a spectropolarimeter (Model J-810, Jasco Co., Japan) at 288 K. The scanning rate was 100 nm/min and the response time was 1.0 s. The α-helical contents in HSA were computed by the value of mean residue ellipticity ($[\theta]$) at 222 nm as follows [35,36]:

$$[\theta]_{222} = \frac{\theta_{obs}}{C_p n l \times 10} \quad (2)$$

$$\alpha\text{-}helix(\%) = \frac{[\theta]_{222} + 3000}{36000 + 3000} \times 100 \quad (3)$$

where θ_{obs} is the observed ellipticity signal, n is the number of peptide bonds and it is 584 in this work, l is the length of quartz cuvette and it is 1.0 cm in this work, and C_p is the molar concentration of protein.

2.5. Molecular Docking Method

The molecular docking between HSA and cleviprex was generated by Molegro Virtual Docker 2008 (v. 3.0). The crystal structure of HSA in the complex with cleviprex was taken from the Research Collaboratory