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Spectroscopic investigation on the food components–drug interaction: The influence of flavonoids on the affinity of nifedipine to human serum albumin

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ABSTRACT

Nifedipine (NDP) is used extensively for the clinical treatment of a number of cardiovascular diseases. Herein, the interaction between human serum albumin (HSA) and NDP and the influence of flavonoids, rutin and baicalin, on their binding properties were investigated in vitro by means of fluorescence and absorption spectroscopy. The fluorescence of HSA was quenched remarkably by NDP and the quenching mechanism was considered as static quenching by forming a complex. The results of thermodynamic parameters indicate that both hydrogen bonds and hydrophobic interactions play the main role in the binding process and the binding process was spontaneous. The binding distance between the amino acid residue of HSA and NDP is 2.608 nm, which indicates that the energy transfer from HSA to NDP can occur with high probability. The decreased association constants and the increased binding distance of NDP binding to HSA in the presence of flavonoids were both due to their competitive binding to the site I of HSA. The results obtained from synchronous fluorescence and three-dimensional fluorescence spectra showed that the interaction between HSA and NDP caused the conformational changes of HSA and the synergism effects of NDP and flavonoids induced the further conformational changes of HSA.

1. Introduction

Flavonoids are a group of natural polyphenols with the structure of diphenylpropane ($C_6C_3C_6$) skeleton that are widely distributed in many plant foods, such as fruits, vegetables, nuts, seeds, grains and tea (Kandaswami et al., 2005; Sankari et al., 2014). According to the structural difference, flavonoids can be classified as flavones, flavonols, flavanones, isoflavones, catechins or flavanols, anthocyanidins, and so on (Ross and Kasum, 2002). Flavonoids in plants are usually combined with sugar into glycosides, and a small number of them exist in the form of aglycones. Rutin (Table 1), a flavonol, is the glycoside between the quercetin and the disaccharide rutinose (α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose). It is a citrus flavonoid glycoside found in many plants (Chua, 2013). Baicalin (Table 1), a flavone, is the glucuronide of baicalein. It is a major component of Chinese medicinal herb *Scutellaria baicalensis* (Huang-chin) (Chen et al., 2014). Rutin is known to exhibit multiple pharmacological activities including antioxidant, anti-inflammation, anti-microbial, anti-tumor and anti-asthma (Chua, 2013; Koval'skii et al., 2014). Similarly, baicalin has been reported to show widely pharmacological and therapeutic properties including anti-inflammatory, anti-adipogenesis, anti-asthma, anti-tumor, anti-bacterial and anti-diarrhea (Chen et al., 2014; Cui et al., 2014; Ma et al., 2014). Because of the potential beneficial effects of flavonoids on human health, foods rich in flavonoids have attracted great interest recently (Kozłowska and Szostak-Wegierek, 2014; Xiao et al., 2014).

In the circulatory system, most drugs are transported as complexes with plasma proteins (Tang et al., 2013). It is well known that the bound drugs will act as a depot, and only free drugs can cross the biological membrane to produce pharmacological effects (Seedher and Bhatia, 2006; Yu et al., 2011). Hence, understanding the mechanism and the related parameters of the interaction between drugs and plasma proteins, such as the number and location of binding sites and binding constant, has an important role in helping not only to know the transport and distribution of drugs in the body, but also to clarify the action mechanism, pharmacokinetics and toxicity of drugs (Khan et al., 2012; Omran et al., 2012; Rub et al., 2014; Xu et al., 2012). Usually, simultaneous binding of various endogenous or exogenous compounds will change the affinity

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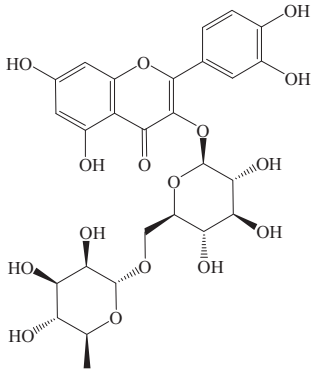
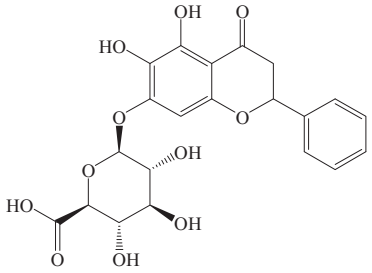
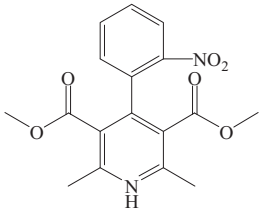
0278-6915/

Table 1
Structural formula of rutin, baicalin and nifedipine.

Name	Molecular formula	Structural formula
Rutin	$C_{27}H_{30}O_{16}$	
Baicalin	$C_{21}H_{18}O_{11}$	
Nifedipine	$C_{17}H_{18}N_2O_6$	

of plasma proteins toward a drug because of the overlap of binding sites or conformational changes (Poór et al., 2013; Sattar et al., 2012; Zohoorian-Abootorabi et al., 2012). Therefore, the detailed investigations of drug–plasma proteins interactions in the presence of other endogenous or exogenous compounds play a dominant role for the interpretation of drug–drug interactions.

Nifedipine (NDP, Table 1), dimethyl 1,4-dihydro-2,6-dimethyl-4(2-nitrophenyl)pyridine-3,5-dicarb oxylate, is a dihydropyridine derivative that belongs to the calcium-channel blockers family. It is used extensively for the clinical treatment of a number of cardiovascular diseases such as essential hypertension, congestive heart failure, and cerebral ischemia (Baghayeri et al., 2013; Gaichore and Srivastava, 2013; Maafi and Maafi, 2013). NDP can rapidly lower blood pressure. Its adverse effects include lethargy, bradycardia, marked hypotension, loss of consciousness, etc. (Poole-Wilson et al., 2006). It is well known that many drug–drug interactions may increase the risk of side effects from occurring. For example, patients taking NDP with other anti-hypertensive drugs can cause low blood pressure. Concurrent administration of cimetidine and NDP may result in alterations in heart rate and blood pressure (Schwartz et al., 1988). Imatinib can increase the effect and toxicity of NDP. Hence, the patients under combined treatment with NDP and some drugs must pay attention to adjust the dosage to minimize resultant adverse health effects. In addition, patients should be warned not to consume anything containing grapefruit or grapefruit juice, as they raise blood levels of NDP (Odou et al., 2005), which indicates that interactions may also exist between drugs and foods. It has been reported that the polyphenols of average human dietary intake is about 1 g/day, with two-third being flavonoids (Scalbert and Williamson, 2000). Up to now, there are only few studies on the influence of flavonoids on drugs–plasma proteins interaction (Mohseni-Shahri et al., 2014; Shi et al., 2012; Zhang et al., 2011). To our knowledge, the influence of flavonoids on NDP binding plasma proteins has still not been investigated.

Herein, human serum albumin (HSA) was selected as the model of plasma proteins because it is the most abundant protein in plasma and has a well-known primary structure. The binding properties of NDP to plasma proteins in vitro in the presence and absence of two kinds of flavonoids, rutin and baicalin, were investigated under simulative physiological conditions by means of fluorescence spectroscopy. It is expected that this work cannot only provide useful information for appropriately understanding the binding mechanism between NDP and plasma proteins at a molecular level, but also is useful in illustrating the interactions between food components and drugs.

2. Experimental procedures

2.1. Materials

HSA was obtained from Sigma-Aldrich Co., USA. The HSA stock solution, 2.00×10^{-5} mol/L, was prepared in 0.05 mol/L Tris–HCl buffer solution of pH = 7.40 containing 0.05 mol/L NaCl, and then stored at 273–277 K in a refrigerator. NDP (purity, >98%) was obtained from Wuhan DKY Technology 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 (purity, >98%) and baicalin (purity, >98%) were obtained from Chengdu Superman Plant & Chemical Development Co., Ltd. China. The stock solutions of NDP, 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 the other materials were of analytical reagent grade and used without further purification. Doubly distilled water was used to prepare solutions. All the solutions were prepared at room temperature and the changes of concentration with temperature were ignored.

2.2. Fluorescence spectral measurements

The fluorescence emission spectra and synchronous fluorescence spectra were carried out on an F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Japan). The specified temperatures were controlled by an SC-15 digital aqueous thermostat (Shanghai Bilon Instrument Co., Ltd., China). In nine volumetric flasks, the final concentration of HSA was 1.00×10^{-5} mol/L, and the final concentration of NDP was changed from 0.0 mol/L to 8.0×10^{-5} mol/L at 1.0×10^{-5} mol/L intervals. The solutions were let to stand for 5 min at room temperature, and then to react for 30 min at specified temperatures. Fluorescence quenching spectra at different temperatures (290, 301 and 310 K) were obtained at an excitation wavelength of 280 nm, with the slit widths of both the excitation and emission set at 5.0 nm and the scanning speed is 1200 nm/min. The fluorescence intensity was corrected for absorption of exciting light and re-absorption of emitted light using the following equation (Anbazhagan and Renganathan, 2008):

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

where F_{cor} and F_{obs} are the fluorescence intensity corrected and observed, respectively, and A_{ex} and A_{em} are the absorbance of system at the excitation and emission wavelengths, respectively. The intensity of fluorescence was corrected in this work. The synchronous