doi:10.46793/ICCBI21.363M

INFLUENCE OF QUERCETIN ON THE BINDING OF TIGECYCLINE TO HUMAN SERUM ALBUMIN

Emina Mrkalić[1], Marina Ćendić Serafinović[2], Ratomir Jelić[3], Stefan Stojanović[3], Miroslav Sovrlić[3]
[1] Institute for Information Technologies
University of Kragujevac, Jovana Cvijića bb, 34000 Kragujevac, Serbia
e-mail: emina.mrkalic@pmf.kg.ac.rs
[2] Faculty of Science,
University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia
e-mail: marina.cendic@pmf.kg.ac.rs
[3] Faculty of Medical Sciences,
University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Serbia
e-mail: rjelic@uni.kg.ac.rs, sstojanovic1987@gmail.com, msovrlic@medf.kg.ac.rs

Abstract

Serum albumin is the major soluble protein in the circulatory system of humans. The metabolism of drugs, their distribution, free concentration, and efficacy depend on the drug-serum albumin interaction [1]. Accordingly, it is important to study the interactions of drugs with serum albumin, which determines the pharmacology and pharmacodynamics of drugs. Quercetin (QUE), a natural polyphenol widely distributed in many plant foods, such as fruits, vegetables, nuts, seeds, grains, and tea [2], bind to serum albumin [3]. Tigecycline (TGC), is a tetracycline antibiotic widely used in the treatment of bacterial infections [4]. This study aimed to investigate the binding properties of TGC to HSA in the presence of QUE, under physiological conditions, by fluorescence spectroscopy.

Keywords: human serum albumin, interactions, tigecycline

1. Introduction

Human serum albumin (HSA), the most abundant protein in plasma. Crystal structure analysis indicates that hydrophobic cavities in subdomains IIA and IIIA in albumin are the principal regions of ligand binding sites for aromatic and heterocyclic drugs [5,6].
Tigecycline (TGC), is a tetracycline antibiotic medication for several bacterial infections [4]. We have been reported interaction between TGC and HSA in aqueous solution [7]. In the present paper, our plans focused on the inclusion and testing effects of flavonoids on the binding of TGC to HSA.
Quercetin is one of the most abundant flavonoids in the human diet. It's an extensively studied molecule because of its complex mechanism of action and numerous pharmacological effects in the human organism [8]. Besides its antioxidant properties, it also displays anti-carcinogenic, anti-inflammatory, cardioprotective, neuroprotective and anti-atherogenic activities [9].

In this study, the quercetin (QUE) was selected to examine their effect on drug binding (TGC) to HSA, as well as the effect on the structure of the active site and the nature of interactions to find out how foods rich in these compounds can affect the use of drugs.

2. Material and Methods

2.1. Materials and solutions

Fatty-acid-free human serum albumin (HSA), tigecycline hydrate (TGC), phosphate-buffered saline (PBS), quercetin (QUE) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA) and used without further purification. HSA (2×10^{-5} M) was dissolved in PBS solution (0.01 M PBS, pH 7.4, 0.002.7 M KCI, 0.137 M NaCl). The stock solution of TGC (3.413×10^{-4} M) was prepared by dissolving them in ethanol and then diluted to 8.538×10^{-5} M with a solution of PBS. The stock solutions of QUE (1×10^{-3} M) were dissolved in ethanol and diluted with a solution of PBS. The deionized water was used for preparing all solutions.

2.2. Apparatus

2D fluorescence spectra were collected on an RF-1501 PC spectrofluorometer (Shimadzu, Japan) with excitation at 295 nm, using a 150 W Xenon lamp source, 1.0 cm quartz cells and a thermostatic bath. Fluorescence spectra were recorded at 298 K in the range of 300-450 nm. The widths of the excitation and emission slit widths were both fixed at 10 nm.

Synchronous fluorescence spectra were collected on an RF-6000 spectrofluorometer (Shimadzu, Japan) at 298 K. In the case of fluorescence synchronous scan spectra, varying the wavelength interval ($\Delta\lambda$) between 15 and 60 nm to assess the alterations in the microenvironment surrounding the aromatic amino acid residues Tyr and Trp, respectively.

3. Results and discussion

Most of the drugs bind with high affinity to one of the two sites, I and II, located in subdomains IIA and IIIA, respectively [5,6]. The presence of the Trp and Tyr amino acids in the structure of HSA causes its intrinsic fluorescence. The intrinsic fluorescence would be quenched even if there is a little change in the local surroundings of the HSA molecule. Here, the effect of QUE on the binding of TGC to HSA was investigated. As already published, the primary binding site of QUE is located in the subdomain IIA [10], indicating that QUE share the same binding sites in the HSA with TGC [7]. The Fig 1A show the fluorescence quenching of HSA-QUE in the presence of varying concentrations of TGC. It can be seen that HSA has a strong fluorescence emission peak around 350 nm, at the excitation wavelength of 295 nm. The increasing of concentrations of TGC, the internal emission fluorescence of the HSA-QUE system gradually decreasing, which indicate the formation of the HSA-TGC-QUE complex. Stern Volmer Equation [11] was used to analyze the quenching mechanism of HSA in the presence of QUE in ternary HSA-TGC-QUE system. Insets on Fig. 1A shows the Stern–Volmer plot for the HSA-QUE fluorescence quenching by increasing concentrations of TGC. The values of K_{SV} obtained from the slopes of the fit lines and k_q for the interaction of TGC with the HSA-QUE system (Table 1). The higher values of K_{SV} for the ternary HSA-TGC-QUE system than that of the binary HSA-TGC system indicate that the presence of QUE enhanced the interaction between HSA and TGC. The values of k_q were greater than 2.0×10^{10} $M^{-1}s^{-1}$, so the probable quenching mechanisms of the intrinsic fluorescence of HSA is a static quenching mechanisms of the intrinsic fluorescence of HSA that originated from the formation of double and triple systems [12]. Further, the binding constants (K_a) and the number of binding sites (n) calculated by the double logarithm equation [11] (Fig.1B) and listed in Table 1. Obtained K_a values of triple complex HSA-TGC-QUE are higher than the double ones (HSA-TGC), which indicate that the binding affinity of TGC to HSA increased in the presence of QUE (non-competitive interference). This simultaneous binding of two drugs led to increased the accessibility of the existing binding sites and hence increase the binding affinity of TGC to HSA. The results showed that TGC could be stored and transported better by HSA in the presence of QUE. This may lead to a decrease in the free concentration of unbound TGC in plasma.

Synchronous fluorescence of HSA can provide characteristic information around tyrosine (Tyr) and tryptophan (Trp) residues when the scanning wavelength intervals ($\Delta\lambda$) are fixed at 15 nm

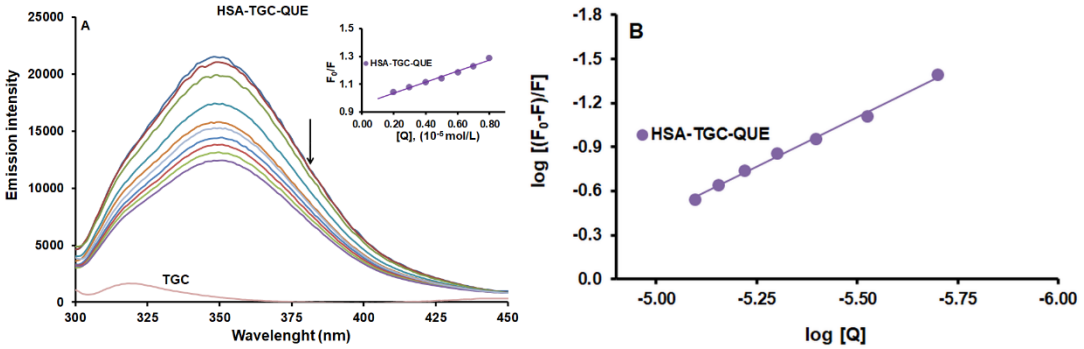

Fig. 1. A) Fluorescence emission spectra of HSA-TGC in the presence of QUE (T = 298 K, pH = 7.4). [HSA] = 2 μM and [QUE] = 2 μM and [TGC] = 0 to 1 x 10^{-5} M. Inset: Stern–Volmer plots of the fluorescence quenching of HSA by TGC in the presence of QUE B) Logarithmic plots of the fluorescence quenching of HSA by TGC in the presence of QUE at 298 K

Table 1 The interaction parameters of the binary (HSA-TGC) and ternary (HSA-TGC-QUE, HSA:QUE = 1:1) system

System [a]	K_{SV} x 10^{-4} [b]	k_q x 10^{-12} [c]	R^2 [d]	K_a x 10^{-5} [b]	n	R^2
HSA-TGC	5.00	5.00	0.996	0.18	0.9	0.991
HSA-TGC-QUE	3.96	3.96	0.9869	22.30	1.35	0.9959

[a] 298 K; [b] M^{-1}; [c] $M^{-1}s^{-1}$; [d] R is the correlation coefficient

and 60 nm, respectively [13]. The red or blue shift in the maximum fluorescence emission wavelength of HSA indicates enhanced hydrophilicity or hydrophobicity of the microenvironment around Tyr or Trp residues, respectively [14]. Fig. 2 shows, with increasing concentrations of TGC, the fluorescence intensity decreased regularly. These results confirm that the fluorescence quenching occurred. Further, there is no shift in the maximum emission wavelength at both 15 and 60 nm, which implies that the binding of TGC does not affect the conformation of the chromophore region [15].

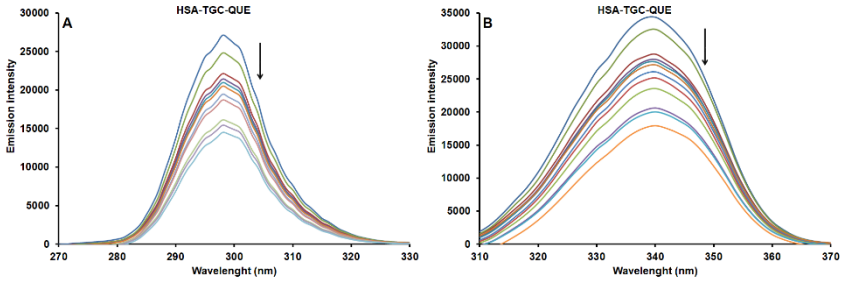

Fig. 2. The effect of QUE on the synchronous fluorescence emission spectra of HSA-TGC system (A: $\Delta\lambda$=15 nm and B: $\Delta\lambda$=60 nm) (T = 298 K, pH = 7.4). [HSA] = 2 μM, [QUE] = 2 μM and [TGC] = 0 to 1 x 10^{-5} M.

4. Conclusion

The studies in this paper reveal that the effect of examined QUE has a positive influence on binding TGC to HSA in simulated physiological conditions by spectroscopic methods. It has been shown through the fluorescence quenching measurements that QUE share IIA subdomain in HSA with TGC drug. Also, there is no significant change in the microenvironment on the Trp residues in the IIA subdomain and there is hydrophilicity or hydrophobicity around it.

Acknowledgments This work was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (Contract Nos. 451-03-9/2021-14/200378, 451-03-9/2021-14/200111 and 451-03-9/2021-14/200122).

References

[1] M. Ishtikhar. G. Rabbani. R.H. Khan, *Interaction of 5-fluoro-5'-deoxyuridine with human serum albumin under physiological and non-physiological condition: A biophysical investigation*, Colloid Surf. B 123 (2014) 469-477.
[2] C. Kandaswami, L.T. Lee, P.P. Lee, J.J. Hwang, F.C. Ke, Y.T. Huang, et al., *The antitumor activities of flavonoids*, InVivo 19 (2005) 895–909.
[3] T.J. Mabry, K.R. Markham, M.B. Thomas, Springer-Verlag, Berlin, Heidelberg, New York. 1970.
[4] N. Kasbekar, *Tigecycline: a new glycylcycline antimicrobial agent*, Am J Health Syst Pharm. 63 (13) (2006) 1235–1243.
[5] G. Sudlow, D.J. Birkett, D.N. Wade, The Characterization of Two Specific Drug Binding Sites on Human Serum Albumin, Mol. Pharmacol. 11 (1975) 824-832.
[6] G. Sudlow, D.J. Birkett, D.N. Wade, *Further characterization of specific drug binding sites on human serum albumin*, Mol. Pharmacol. 12 (1976) 1052–1061.
[7] S.D. Stojanovic, S.M. Jankovic, Z.D. Matovic, I.Z. Jakovljevic, R.M. Jelic, Interaction between tigecycline and human serum albumin in aqueous solution, Monatsh Chem 146 (2015) 399-409.
[8] A.V. Anand David, R. Arulmoli, S. Parasuraman, *Overviews of biological importance of quercetin: a bioactive flavonoid*, Pharmacogn. Rev. 10 (2016) 84–89.
[9] A. Crozier, I.B. Jaganath, M.N. Clifford, *Dietary phenolics: chemistry, bioavailability and effects on health*, Nat. Prod. Rep. 2009, 26, 1001-1043.
[10] S.D. Stojanović, J.M. Nićiforović, S.M. Živanović, J.V. Odović, R.M. Jelić, *Spectroscopic studies on the drug-drug interaction: the influence of fluoroquinolones on the affinity of tigecycline to human serum albumin and identification of the binding site*, Monatsh. Chem. 151 (2020) 999-1007.
[11] J.R. Lakowicz, Principles of fluorescence spectroscopy, 3rd ed. Springer, New York, (2006).
[12] J.-H. Shi, Y.-Y. Lou, K.-L. Zhou, D.-Q. Pan, *Elucidation of intermolecular interaction of bovine serum albumin with Fenhexamid: A biophysical prospect*, Journal of Photochemistry & Photobiology B: Biology 180 (2018) 125–133, DOI: 10.1016/j.jphotobiol.2018.01.025.
[13] L. Zhu, F. Yang, L. Chen, E.J. Meehan, M. Huang, *A new drug binding subsite on human serum albumin and drug-drug interaction studied by X-ray crystallography*, J. Struct. Biol.162 (2008) 40-49.
[14] G.W. Zhang, Q.M. Que, J.H. Pan, J.B. Guo, *Study of the interaction between icariin and human serum albumin by fluorescence spectroscopy*, J. Mol. Struct. 881 (2008) 132–138.
[15] J.H. Shi, K.L. Zhou, Y.Y. Lou, D.Q. Pan, *Multi-spectroscopic and molecular modeling approaches to elucidate the binding interaction between bovine serum albumin and darunavir, a HIV protease inhibitor*, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 188 (2018) 362-371.

ISBN-978-86-82172-01-7