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Interaction of imatinib mesylate with human serum transferrin: The comparative spectroscopic studies

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ABSTRACT

Imatinib mesylate (Imt) is a tyrosine kinase inhibitor mainly used in the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia (Ph + CML). Human serum transferrin is the most abundant serum protein responsible for the transport of iron ions and many endogenous and exogenous ligands. In this study the mechanism of interactions between the imatinib mesylate and all states of transferrin (apo-Tf, Htf and holo-Tf) has been investigated by fluorescence, ultraviolet–visible (UV–vis), circular dichroism (CD) and zeta potential spectroscopic methods. Based on the experimental results it was proved that under physiological conditions the imatinib mesylate binds to the each form of transferrin with a binding constant c.a. 10^5 M^{-1}. The thermodynamic parameters indicate that hydrogen bonds and van der Waals were involved in the interaction of apo-Tf with the drug and hydrophobic and ionic strength participate in the reaction of Htf and holo-Tf with imatinib mesylate. Moreover, it was shown that common metal ions, Zn^{2+} and Ca^{2+} strongly influenced apo-Tf-Imt binding constant. The CD studies showed that there are no conformational changes in the secondary structure of the proteins. No significant changes in secondary structure of the proteins upon binding with the drug and instability of apo-Tf-Imt system are the desirable effects from pharmacological point of view.

1. Introduction

Human serum transferrin is a monomeric glycoprotein with a molecular weight of about 80 kDa. It contains two similar, but not identical metal-binding sites located in its N- and C-terminal domains [1]. Transferrin (Tf) combines with two Fe^{3+} ions (each domain of Tf binds one Fe^{3+}) in the presence of bicarbonate (or carbonate) ions which are anchored in place by a conserved arginine residue (Arg124 in the N-lobe and Arg456 in the C-lobe). This reaction is pH dependent: it is maximal between pH 7.5–10, but partial dissociation occurs at pH 6.5, and complete dissociation at pH 4.5. It has also been shown that for each Fe^{3+} ion bounds to the protein, one bicarbonate ion is concomitantly bounded and three protons are released [2]. Moreover, in both Tf lobes the Fe^{3+} is coordinated by identical ligands: two tyrosine residues, one aspartic acid and one histidine residue (Tyr95, Tyr188, Asp63 and His249 in the N-lobe; Tyr426, Tyr517, Asp392 and His585 in the C-lobe) [3]. Transferrin plays a very important transport role of metal ions and organic ligands such as drugs. The protein can be used as an effective carrier of anticancer drugs because receptors for this protein, TfR1 and TfR2, are overexpressed at the surface of rapidly proliferating malignant cells, due to the high demand of cancer cells for iron ions [4]. The differic transferrin (holo-Tf) is reported to have a higher affinity for TfR than the monoferric (Htf) and iron-free form (apo-Tf) [5]. At present a conjugate consisting of transferrin that is bound to the highly potent diphtheria toxin through a lysine cross-linker and a thioester (TransMID) [6] undergoes III phase clinical trials to treat the patients with progressive and/or recurrent, non-resectable glioblastoma multiforme [7].

Imatinib mesylate (the chemical name is 4-[(4-methylpiperazin-1-yl)methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide) is an oral medication mainly used for treating Philadelphia positive chronic myeloid leukemia (Ph + CML) and Ph + acute lymphoblastic leukemia (ALL), kit + gastrointestinal stromal tumors (GIST) and many of other cancers. Imatinib mesylate is classified as a protein - tyrosine kinase inhibitor that inhibits the BCR-ABL tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in CML. Imatinib is also an inhibitor of the receptor tyrosine kinases for platelet-derived growth factor (PDGF) and stem cell factor (SCF), c-kit and inhibits PDGF and SCF-mediated cellular events [8]. Kinase inhibitors prevent the growth of tumors by reducing the action of proteins that control cell division, growth and survival. These proteins are usually present in large quantities or are more active in cancer cells. Growth and survival of cancer cells are reduced by reducing the activity of these proteins. The pharmacokinetic of the drug is similar in CML and GIST patients. Imatinib is well absorbed after oral administration with maximum concentration achieved within 2–4 h post-dose. Mean absolute

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bioavailability is 98%. Following oral administration, the elimination of half-lives of imatinib and its main active metabolite, the *N*-demethylated piperazine derivative, formed predominantly by CYP3A4, the major enzyme responsible for metabolism of imatinib, are approximately 18 and 40 h, respectively. Imatinib elimination is predominately in the feces, mostly as metabolites [8].

The interactions of a drug molecule with the proteins play an important role in drug pharmacokinetics, pharmacology and influence the absorption, distribution, metabolism and excretion properties of them. Moreover, the binding processes have an effect on the therapy effectiveness, because only unbounded fraction of the drug shows the therapeutic effect. The studies on this aspect can provide very useful information of the nature and binding process between drugs and serum proteins that determine the therapeutic effectivity of the medicaments.

In the present study the interactions between transferrin in all states with imatinib mesylate have been investigated using spectroscopic methods. There are many methods such as UV–visible [9], fluorescence [10,11] and circular dichroism (CD) spectroscopy [12], equilibrium dialysis [13] and potentiometry [14,15] to investigate the binding of ligands to proteins. Among these methods, equilibrium dialysis is used widely, but it requires the analysis of free and total drug concentration and takes a long time. In potentiometric method, ion selective electrodes are used. These electrodes have the lack of selectivity for many ligands such as drug molecules [14,15]. Fluorescence techniques are very helpful in protein-drug interactions due to their high sensitivity, rapidity and ease of implementation. The fluorescence measurements can provide some useful information about the binding of small molecules to protein such as binding mechanism, binding mode, binding constants and binding numbers [16]. Based on the fluorescence techniques the binding constants, sites and thermodynamic parameters of the Tf-Imt systems have been obtained. The quenching mechanism has been analysed by the Stern-Volmer equation. The conformational changes of the proteins have been determined by synchronous fluorescence and circular dichroism spectroscopy. The effect of metal ions on the binding constant of apo-Tf-Imt was also examined. Moreover, the zeta potential experiments were done to determine the stability of apo-Tf-Imt adducts depending on the pH and drug concentration.

2. Materials and Methods

2.1. Materials and Solutions

All reagents were of analytical grade and purchased from Sigma-Aldrich Co. (apo-transferrin human, Catalog No. T1147, ≥98% purity, holo-transferrin human, Catalog No. T0665, ≥97% purity, transferrin human, Catalog No. T8158, ≥98% purity; imatinib mesylate, Catalog No. SML1027, ≥98% purity). The transferrin solutions (1.00×10^{-5} M) were prepared in phosphate buffer of pH = 7.40, citrate buffer of pH = 3.50 or 5.50 and Tris-HCl buffer of pH = 8.50. An imatinib mesylate (4.20×10^{-4} M) and metals solutions (1.10×10^{-4} M) were prepared in double distilled water. The measured samples were prepared by mixing a quantity of imatinib mesylate with protein and metal solution and then diluted by proper buffer. The samples solutions were incubated at 303 K and 310 K for 15 min before each experiment.

2.2. Apparatus

2.2.1. UV–Vis Spectroscopy

UV–vis absorption spectra were obtained with a Lambda 20 spectrophotometer. The absorption measurements of all samples were carried out using quartz cells with a 1-cm optical path. All measurements were performed after incubation samples at 303 K and 310 K for 15 min.

2.2.2. Fluorescence Spectroscopy

Fluorescence measurements were performed on a Hitachi F-7000 spectrofluorimeter with a 150 W Xenon lamp, using a 1.0-cm quartz cell. The widths of excitation and emission slit were set at 5.0 nm. The excitation wavelengths were set at 280 nm and 295 nm, and the emission wavelength was recorded between 290 and 500 nm. The fluorescence intensities were corrected for inner filter and dilution effects

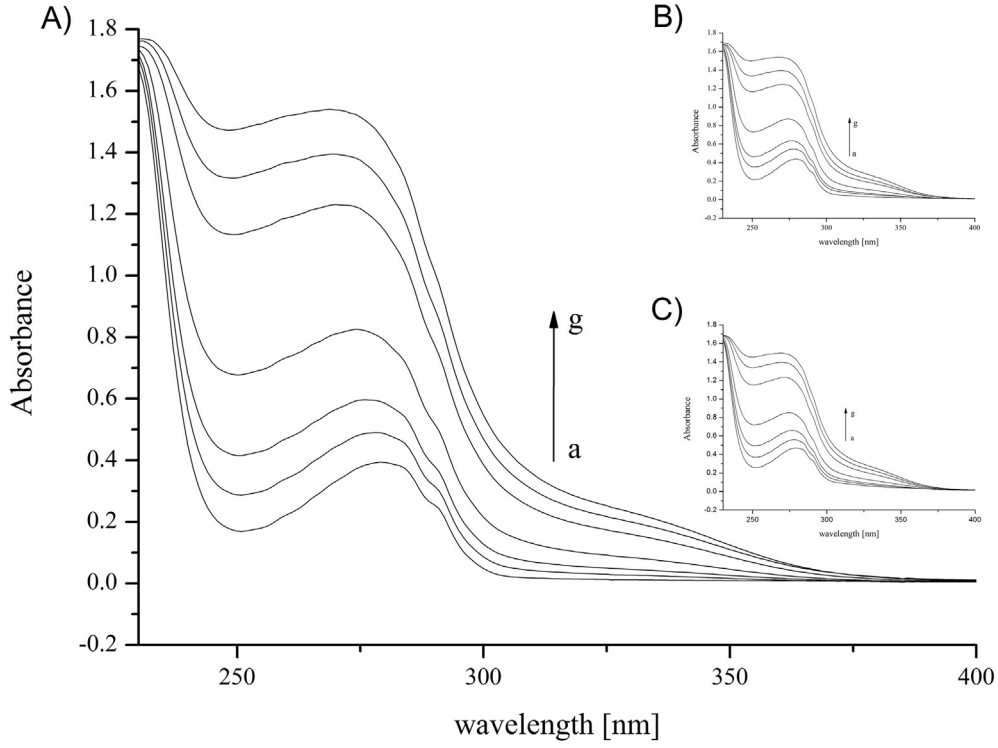

Fig. 1. Absorption spectra of the apo-Tf-Imt system (A) (T = 310 K, pH = 7.40). Line (a–g) the ratios of $C_{Imt}/C_{protein}$ = 0, 1, 2, 4, 8, 10, 12; $C_{protein}$ = 5 μM. Inset: absorption spectra of the Htf-Imt (B) and holo-Tf-Imt (C) systems at the same conditions.