ARS Pharmaceutica
ISSN: 0004-2927
http://farmacia.ugr.es/ars/

ARTICULO ORIGINAL

Interaction of palmitic acid with losartan potassium at the binding sites of bovine serum albumin

Ferdosi Kabir A[1], Nazim Uddin K[2], Nazmus Sadat A.F.M[1]., Hossain Mahboob[1] and Abdul Mazid Md[2] *

1Department of Pharmacy, The University of Asia Pacific, Dhaka-1209, Bangladesh

2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

mazid_ma@hotmail.com Tel:+880-2-8612069

ABSTRACT

The binding of losartan potassium, an angiotensin II receptor antagonist, to bovine serum albumin was studied by equilibrium dialysis method (ED) in presence or absence of palmitic acid. The study was carried out using ranitidine and diazepam as site-1 and site-2 specific probe, respectively. Different analysis of binding of losartan to bovine serum albumin suggested two sets of association constants: high affinity association constant (k1 = 11.2 x 105 M-1) with low capacity (n1 = 2) and low affinity association (k2 = 2. 63 x 105 M-1) constant with high capacity (n2 = 10) at pH 7.4 and 27°C. During concurrent administration of palmitic acid and losartan potassium in presence or absence of ranitidine or diazepam, it was that found that palmitic acid causes the release of losartan potassium from its binding site on BSA resulting reduced binding of losartan potassium to BSA. The increment in free fraction of losartan potassium was from 13.1% to 47.2 % upon the addition of increased concentration of only palmitic acid at a concentration of 0 x 10-5 M to 16 x 10-5 M. In presence of ranitidine or diazepam as site specific probes, palmitic acid further increases the free fraction of losartan potassium were from 22.8% to 53.4% and 35.3 to 65.5%, respectively. This data provided the evidence of interaction of higher concentration of palmitic acid at the binding sites on BSA changing the pharmacokinetics properties of losartan potassium.

KEYWORDS: Losartan potassium, palmitic acid, bovine serum albumin, equilibrium dialysis, binding sites

INTRODUCTION

Serum albumin, the most abundant protein in blood plasma serves as a depot and transport protein for numerous endogenous and exogenous compounds[1]. The most outstanding property of albumin is its ability to bind reversibly an incredible variety of ligands including fatty acids, amino acids (tryptophan and cysteine), steroids, metals such as calcium, copper and zinc, and numerous pharmaceuticals [2].

On the basis of probe displacement method, it has been found that there are at least three relatively high specific drug binding sites on the Human serum albumin (HAS) molecule. These sites are commonly called the warfarin/ranitidine, the benzodiazepine and the digoxin binding sites, and are also denoted as site I, site II and site III, respectively[3,4]. Site II or benzodiazepine binding site is more specific than site I. Most drugs bind with proteins by a reversible process. Plasma protein binding properties are primarily determinants of the pharmacokinetic properties of most of the drugs, such as plasma clearance, half-life, apparent volume of distribution and the duration and intensity of pharmacologic effect[5]. Drug displacement also affects other aspects of drug deposition, such as, metabolism and excretion [6].

Free fatty acids are highly protein bound and replace many drugs and other ligands from its' binding site on albumin [7-9]. Goodman [10] concluded that the two sites in the first binding class of fatty acids (laurate, myristate, palmitate, stearate, oleate and linoleate), in contrast to the sites in the second binding class, are "rather specifically constructed". The specificity of the high affinity sites of long-chain fatty acids was supported by some authors [11]. Other binding studies indicate that the high affinity binding sites of short and medium-chain fatty acids probably also are placed in other regions on the albumin molecule [7].

Losartan potassium, an angiotensin II receptor antagonist and commonly prescribed for the management of hypertension. A recent study shows that losartan potassium highly protein bound and suprapharmacologic concentrations of the NSAIDs increased the free fraction of losartan [13]. However, until now, no report has been published relating the interference of free plasma fatty acid in the binding profile of losartan potassium. Consumption of high fat diet sharply increases the free fatty acid levels in blood. Since fatty acids are highly protein bound, therefore, we hypothesize that high dietary fat originated free fatty acid may displace many drugs from its binding sites on albumin and may affect the pharmacokinetic properties of the drugs. Therefore, the present study was undertaken to characterize the binding profile of losartan potassium as well as to notify the interaction of palmitic acid with losartan potassium at its binding site on albumin using bovine serum albumin employing equilibrium dialysis methods.

MATERIAL AND METHODS

Materials and instruments

Dialysis membrane (molecular cut off 3500 daltons) and bovine serum albumin (fatty acid free, fraction V, 96-98%) used in the experiment were purchased from Medicell International Ltd., UK and Sigma Chemical Co., USA, respectively. Losartan potassium, ranitidine hydrochloride and diazepam were kind gift from a local pharmaceutical company of Bangladesh. Disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), palmitic acid and borax ($NaB_4O_7.H_2O$) were obtained from Sigma., USA. High-resolution UV-VIS spectrophotomer (UV-1800, Shimadzu, Japan) and Metabolic Shaking Incubator (Clifton Shaking Bath, Nickel Electro Ltd., England.) were used in the experiment.

All other chemicals used in the experiment were commercial grade. Equilibrium Dialysis method was employed in this study [14,15]. Dialysis membrane used in the experiment was cut into small pieces and was boiled for 8 hours at 65-70 °C in de-ionized water to remove sulfur.

Preparation of Standard Curve

For the preparation of standard curves of ranitidine hydrochloride, diazepam and losartan potassium, solutions of different concentrations (0×10^{-5} M to 20×10^{-5} M) of these drugs were prepared in phosphate buffer of 7.4 and taking absorbance values at determined λ_{max} 318 nm, 235 nm and 273 nm respectively. Standard curves were obtained by plotting the absorbance values against the corresponding the concentrations.

Estimation of association constant

To determine the association constant of losartan potassium, different concentrations (2×10^{-5} M to 20×10^{-5} M) of losartan potassium solutions were mixed with prepared BSA solution (2×10^{-5} M in phosphate buffered saline, pH 7.4) to get a final volume of 5 ml each. These solutions were allowed to stand for sometime for the maximum binding of losartan potassium to BSA. From each mixture, 3.5 ml of solution was withdrawn poured into previously prepared semi-permeable membrane tubes and both sides of the membranes were sealed properly as there was no leakage. The membrane tubes containing drug-protein mixture were immersed in conical flasks containing 30 ml of phosphate buffer (pH 7.4) and were placed in a metabolic shaker for dialysis for 12 hours (27 °C, 20 rpm). Buffer samples were collected from each conical flask after dialysis and free fraction of losartan potassium was measured by UV spectrophotometer (λ_{max} 273 nm).

Determination of binding site of losartan potassium using ranitidine as site-I specific probe

To determine the binding sites of losartan potassium at BSA, the concentrations of BSA and probe (ranitidine hydrochloride as site-I specific probe) were remained fixed in 1:1 ratio (2×10^{-5} M: 2×10^{-5} M) and the concentration of losartan potassium was added in increased concentration (0 to 16×10^{-5} M). So, the final ratio of BSA: Probe: losartan potassium were 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:5, 1:1:6, 1:1:8. The dialysis was carried out as described above and free fraction of ranitidine hydrochloride was measured at λ_{max} 318 nm. Only BSA solution and mixture of BSA and losartan potassium were used positive and negative control during the measurement. Alternatively, BSA and losartan potassium were mixed in 1:1 ratio (2×10^{-5} M: 2×10^{-5} M) and ranitidine hydrochloride was added in increasing concentration (0 to 16×10^{-5} M) and the final ratio of BSA: Drug: Probe were 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:5, 1:1:6, 1:1:8. The dialysis was carried out as described above and free fraction of losartan potassium was measured at λ_{max} 204 nm. As describe above, negative and positive control were maintained during the measurement.

Determination of binding site of losartan potassium using diazepam as site-II specific probe

The binding sites of losartan potassium at BSA, using diazepam as site-II specific probe, were determined employing the same methods as describe above. Briefly, BSA: diazepam: losartan potassium were mixed at 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:5, 1:1:6, 1:1:8. The dialysis was carried out as described above and free concentration of diazepam was measured at λ_{max} 235 nm. Alternatively, BSA: losartan: diazepam were taken as 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:5, 1:1:6, 1:1:8. After dialysis, free concentration of losartan potassium was measured at λ_{max} 204 nm. Negative and positive controls were maintained in both cases of measurements.

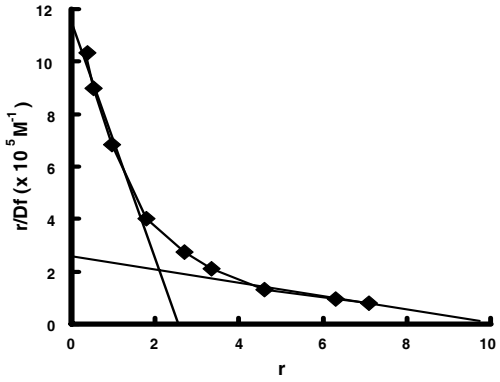
Effect of palmitic acid on losartan potassium bound to BSA

The effect of palmitic acid on losartan potassium, when bound to BSA was estimated in absence and in presence of site specific probes using ranitidine as site-I specific and diazepam as site-II specific probes, respectively. In absence of site specific probes, the BSA and losartan potassium was mixed at 1:1 ratio (2 x10^{-5} M: 2 x 10^{-5} M) and then palmitic acid was added in increasing concentration (0 to 16 x 10^{-5} M) to make final ratio of BSA, losartan and palmitic acid in each experiment as 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:6, 1:1:8 and 1:1:10. While in presence of probes; BSA, probe and losartan potassium were mixed at ratio of 1:2:1 and palmitic acid was added in increasing concentration to make the final ration of protein, probe, losartan and palmitic acid as 1:2:1:0, 1:2:1:1, 1:2:1:2, 1:2:1:4, 1:2:1:6, 1:2:1:8 and 1:2:1:10. Dialysis was carried out and the amount of free losartan potassium was measured in absence and in presence of probes as described above. Only BSA was used as positive control, mixture of BSA and palmitic acid, and mixture of BSA, palmitic acid and probe were used as negative control.

RESULTS

Estimation of binding parameters

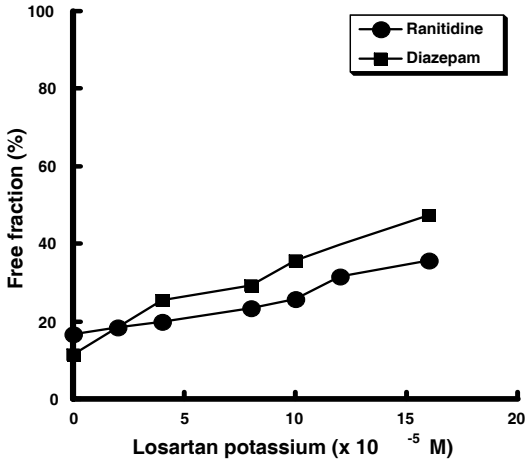
Figure l. Scatchard plot for the binding of losartan potassium to BSA at $p^{H\,7.4}$ and 27 °C. losartan potassium [0 x 10^{-5} M to 20 x 10^{-5} M] was added to BSA [2 x 10^{-5} M] and dialyzed as described under materials and methods. Free fraction of losartan was measured by UV-spectroscopic method and analyzed.



The binding parameters of losartan potassium have been characterized using Scatchard type of analyses of the binding of drug to albumin (Figure 1). Scatchard analysis of the binding of the drug at pH 7.4 and at 27° C provided a non-liner curve, suggesting the presence of at least two classes of binding sites for the binding of losartan potassium to BSA. As shown in Figure 1, the number of high affinity binding site (n_1) for losartan was approximately two (low capacity) and the number of low affinity binding site (n_2) was approximately 10 (high capacity). The high affinity association constant (k_1) for the losartan binding to BSA at pH 7.4 is quite high (11.2 x 10^5 M^{-1}), while the low affinity association constant (k_2) for this drug to BSA is about 4 fold lower (2.63 x 10^5 M^{-1}) than that of primary association constant. These findings indcate that losartan are highly bound to BSA.

Interaction of losartan potassium with site specific probes

Figure 2. Free fraction of ranitidine (●) or diazepam (■) bound to BSA (1:1) upon the addition of losartan potassium at 27°C and pH 7.4. The concentrations used in the binding study were: [BSA] = [ranitidine] = 2×10^{-5} M; [BSA] = [diazepam] = 2×10^{-5} M; and [losartan] = 0-16 $\times10^{-5}$ M.



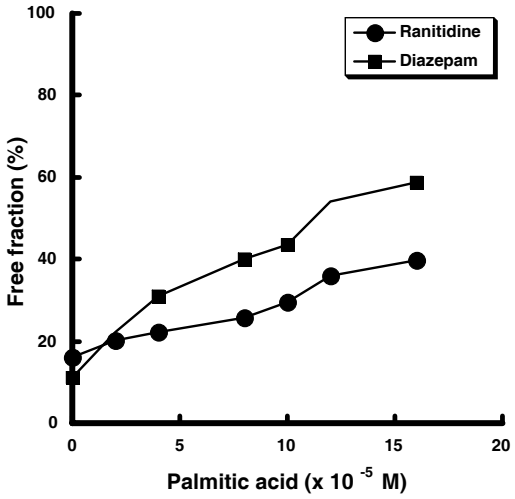
The effects of losartan potassium on the binding of site specific probes were examined to determine whether losartan potassium binds preferentially with site-I or site-II on BSA.As mentioned under materials and methods, site specific probe and BSA were mixed at 1:1 molar ratio and losartan potassium was added in an increased concentration from 0 x 10^{-5} M to 16 x 10^{-5} M. The results showed that losartan potassium cause the increment of free fraction of ranitidine and diazepam from 16.6% to 35.7% and 11.5% to 47.3%, respectively (Figure 2).

Interaction of palmitic acid with site specific probes

The effect of palmitic acid was measured to know whether it can release the ranitidine and diazepam from their binding sites or not. It was found that palmitic acid at a concentration

from 0 x 10^{-5} M to 16 x 10^{-5} M increased the free fraction ranitidine from 16.0 to 39.8% and of diazepam from 11.5% to 58.7% (Figure 3).

Figure 3. Free fraction of ranitidine (●) or diazepam (■) bound to BSA (1:1) upon the addition of palmitic acid at 27°C and pH 7.4. The concentrations used in the binding study were: [BSA] = [ranitidine] = 2×10^{-5} M; [BSA] = [diazepam] = 2×10^{-5} M; and [losartan] = 0-16 $\times10^{-5}$ M.



Interaction of palmitic acid with losartan potassium at the binding sites on BSA

Figure 4: Free fraction of losartan potassium in the absence or presence of ranitidine as site I specific probe to BSA at 2:1 molar ratio upon the addition of palmitic acid at 27 °C and pH 7.4. The molar concentrations used in the binding study were: [BSA]:[probe]:[losartan] = 1:2:1; and [palmitic acid] = 0-16 $\times10^{-5}$ M.

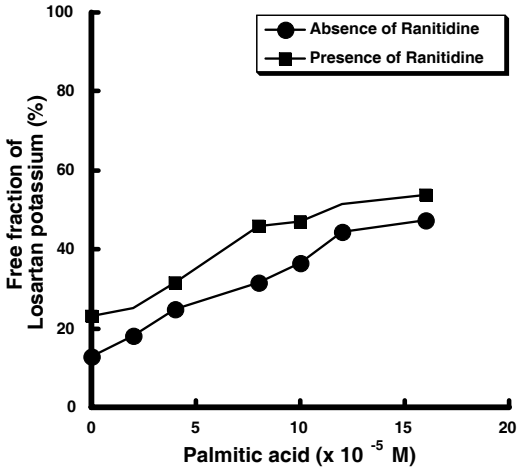
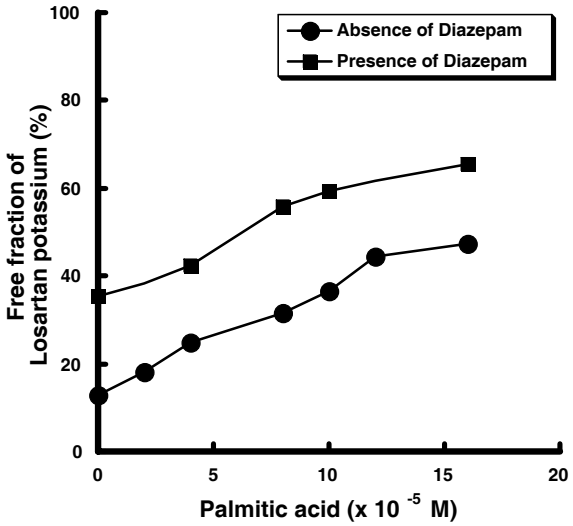


Figure 5: Free fraction of losartan potassium in the absence or presence of diazepam as site II specific probe to BSA at 2:1 molar ratio upon the addition of palmitic acid at 27 °C and pH 7.4. The

molar concentrations used in the binding study were: [BSA]:[probe]:[losartan] = 1:2:1; and [palmitic acid] = 0-16 $\times 10^{-5}$ M.



The interactions at bindings sites on BSA were measured between losartan potassium and palmtic acid in the absence or in presence of site specific probes ranitidine and diazepam, respectively. In absence of both ranitidine and diazepam, palmitic acid increased the free fraction of losartan potassium from 13.1% to 47.2% with the addition of palmitic acid from 0 x 10^{-5} M to 16 x 10^{-5} M (Figure 4 and 5). Whereas, in presence of ranitidine and diazepam as site specific probes, palmitic acid at the same concentration, incremented the free fraction of losartan potassium from 23.0% to 53.5% and 35.6% to 65.5%, respectively.

DISCUSSION

Binding of drugs are determined by studying its ability to displace the site specific probes. In this study, ranitidine hydrochloride and diazepam were used as site-I and site-II specific probes, respectively. The association constants as shown in Table 1 indicate that losartan potassium is highly bound to BSA. Figure 2 shows the change in free concentration of ranitidine and diazepam by losartan. It is seen that the free concentration of ranitidine increased from 16.6% to 35.7%, whereas, the free concentration of diazepam was increased from 11.5% to 47.3% by the same drug. From this observation it can be said that losartan potassium at higher concentration displaced diazepam to a greater extent as compared to ranitidine, so losartan has greater affinity for site II than for site I on the BSA molecule. This implies the fact that at a lower drug to BSA ratio, losartan binds to its high affinity site i.e., site II or the benzodiazepine site, whereas at higher ratio it not only binds to its high affinity site but also to its low affinity site i.e., site I or the warfarin site on the BSA molecule.

Table 1: Association constant of losartan potassium bound to BSA at pH 7.4 (27 °C)

	Association constant	
p^H	k_1 (high affinity) $\times 10^5 M^{-1}$	k_2 (low affinity) $\times 10^5 M^{-1}$
7.4	11.2 ± 0.07	2.6 ± 0.03

Note: Value represents the mean ± SEM of three independent experiments

The pharmacokinetic properties of drugs are influenced by exogenous as well as endogenous compounds by binding to serum albumin in a reversible manner. Regular diet contains various fats and fatty acids. These fatty acids are highly protein bound and displaces drugs and other endogenous molecules from their binding sites on albumin [10-12]. Here we, for the first time, reported the *in vitro* interaction of palmitic acid with losartan potassium at the binding sites on BSA. Plasma protein binding properties are considered to be the primary determinants of the pharmacokinetic properties of many drugs. Therefore, any alteration or change in the serum albumin binding of these drugs might lead to a change in the pharmacokinetic properties of these drugs. Figures 4 and 5 showed the change in the free concentration of losartan potassium bound to BSA in the presence of palmitic acid at pH 7.4 and at 27°C. As observed in Figure 4, the free fraction of losartan potassium bound to BSA was increased from 13.1% to 47.2% by palmitic acid in the absence of ranitidine, a site I specific probe. Whereas, in the presence of ranitidine, this increment was from 23.0% to 53.5%. While siteII was blocked by sufficient amount of diazepam, palmitic acid increased the free fraction of losartan potassium from 35.6% to 65.5% as shown in Figure 5. These findings do not contradict with the number of high and low affinity binding sites of losartan potassium on BSA. When site II was blocked by diazepam, initial free fraction was naturally at an elevated level because the number of low affinity binding sites of losartan potassium on BSA was 10 (this means that 10 molecules of losartan potassium bind to site-II on one molecule of BSA). This suggests that losartan potassium is displaced to a greater extent by palmitic acid in the presence of diazepam compared to ranitidine.

CONCLUSION

Serum binding can be one of the determinants for the pharmacokinetics of drugs. The aim of the study was to observe the effects of fatty acid on the binding of losartan potassium, an antihypertensive drugs, to BSA. Interaction at the binding site was also carried out during concurrent addition of fatty acid, Palmitic acid. We found that palmitic acid, a saturated fatty acid, displaces losartan potassium from its binding sites on BSA. As there is strong analogy between BSA and HSA, it is assumed that similar types of binding characteristics will be exhibited by losartan when bound to HSA. Sometimes the pharmacologic activity of a drug is related to its protein binding. If a drug shows less affinity for albumin due to any alteration in protein binding, the pharmacologic effect of the drug may be significantly altered. However, the results of the present study in combination with the current advances in the binding of

losartan potassium and interaction with fatty acid might be helpful in realizing to the overall binding behavior of the drug with HSA. However, from our limited data it is too early to draw such conclusion about the pharmacokinetic/ pharmacological properties of the drug. It deserves a more detailed study using *in vivo* experimental model.

BIBLIOGRAPHY

1. Kragh-Hansen, U. Molecular aspects of ligand binding to serum albumin. J. The American Society for Pharmacology and Experimental Therapeutics. 1981a. 33:17-46.
2. He, X.M. and Carter, D.C. Atomic structure and chemistry of human serum albumin. Nature. 1992, 358:209-215.
3. Sudlow, G., Birkett, D.J. and Wade, D.N. The characterization of two specific binding sites on human serum albumin. Mol. Pharmacol., 1975, 11:824-832.
4. Sudlow, G., Birkett, D.J. and Wade D.N. Further characterization of two specific binding sites on human serum albumin. Mol. Pharmacol., 1976, 12:1052-1061.
5. Jiunn, H. L., David, M. C. and Daniel, E. D. Protein binding as a primary determinant of the clinical pharmacokinetic properties of non-steroidal anti-inflammatory drugs. Clinical Pharmacokinetics. 1987, 12, 402-432.
6. Rahman, M. H., Yamasaki, K., Shin,Y. H., Lin, C. C., and Otagirin, M., Characterization of high affinity binding sites of non-steroidal anti-inflammatory drugs with respect to site-specific probes on human serum albumin. Biol Pharm Bull., 1993, 16(11):1169-1174.
7. Kragh-Hansen, U. Effect of aliphatic fatty acids on the binding of Phenol Red to human serum albumin. Biochem. J. 1981b, 195:603-613.
8. Horiuchi, T., Johno, I., Hasegawa, T., Kitazawa. S., Goto, M. and Hata T. Inhibitory Effect of Free Fatty Acids on Plasma Protein Binding of Disopyramide in Haemodialysis Patients. Eur J Clin Pharmacol 1989, 36:175-180.
9. Cunningham, V. J., Gay, L. and Sttoner, H. B. The binding of L-tryptophan to serum albumins in the presence of non-esterified fatty acids. Biochem. J. 1975, 146:653-658.
10. Goodman, D.S. : The interaction of human serum albumin with long chain fatty acid anions. J.Am, Chem Soc. 1958, 80:3892-3898.
11. Spector AA. Fatty acid binding to plasma albumin. J Lipid Res. 1975, 16(3):165-179. Links
12. Ashbrook JD, Spector AA, Santos EC, Fletcher JE. Long chain fatty acid binding to human plasma albumin. J Biol Chem. 1975, 250(6):2333-2338.
13. Christ DD. Human plasma protein binding of the angiotensin II receptor antagonist losartan potassium (DuP 753/MK 954) and its pharmacologically active metabolite EXP3174. J Clin Pharmacol. 1995, 35(5):515-520.