

In vitro Interaction of Metformin Hydrochloride with Levofloxacin and its Influence on Protein Binding

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Abstract

The *in vitro* study of protein binding of metformin hydrochloride and its 1:1 mixture with levofloxacin has been conducted by equilibrium dialysis method at physiological pH and temperature. In this study, the number of binding sites and affinity constants of metformin hydrochloride and its 1:1 mixture with levofloxacin were calculated by Scatchard method. The Scatchard plots revealed that the interaction of metformin hydrochloride with levofloxacin lowered the affinity and decreased the percentage of binding of metformin hydrochloride in the mixture to bovine serum albumin (BSA). Thus, the interaction of metformin hydrochloride with levofloxacin can increase the free drug concentration of metformin hydrochloride in the blood plasma. This may change the pharmacokinetic and pharmacodynamic properties of metformin hydrochloride.

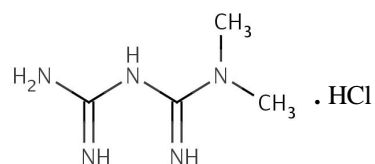
Keyword: Metformin Hydrochloride, Levofloxacin, Protein binding, Bovine serum albumin (BSA), Dialysis membrane

Introduction

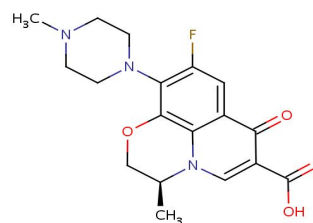
Levofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It acts by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. Metformin hydrochloride is a biguanide antidiabetic. It is slowly and incompletely absorbed from the gastrointestinal tract. The absolute bioavailability of a single 500 mg dose is reported to be about 50-60%, although this is reduced somewhat if taken with food. Once absorbed plasma protein binding is negligible and it is excreted unchanged in the urine. The plasma elimination half-life is reported to range from about 2-6 h after oral doses. Metformin is distributed into breast milk in small amounts (Scheen, 1996; Sambol *et al.*, 1996).

Protein binding is one of the important pharmacokinetic parameters of a drug. After oral administration the drug enters the systemic circulation through absorption and binds with plasma protein. Among plasma proteins, albumin is highly bound to drugs. Other

important plasma proteins are α -globulin and γ -globulin. The interaction of a drug with protein may be reversible or irreversible. In reversible case, the drug-protein complex acts as a reservoir and release the unbound (free) drug and equilibrium exists between bound and unbound fractions



Metformin Hydrochloride



Levofloxacin

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of a drug. Drugs are bound to plasma protein at sites located on the surface of the protein. The idea of binding sites is suggested by the relative sizes of the drugs and proteins. The extent of plasma protein binding is an important parameter of drug action. Binding to plasma protein may have a profound effect on distribution, pharmacological action and rate of elimination.

Drug-drug interaction results when one drug alters the known therapeutic response of other drug that has been administered concomitantly. A common practice in the medical science is the prescription of multi drugs, which may sometimes be neither safe nor effective and may be deleterious (Bari *et al.*, 2000; Amran *et al.*, 2006, 2008).

Most physicians believe that diabetic individuals are predisposed to infections and that infection complicates the control of the diabetes. The chance of infection is a common phenomenon in diabetic compared with nondiabetic patients including infections of the urinary tract, skin and soft tissues. Tuberculosis, once a proven threat to diabetic individuals, is a less serious problem now that effective screening and chemoprophylaxis programs have been initiated. Fluoroquinolones are commonly used in the treatment of tuberculosis (TB) for drug-sensitive patients who are intolerant to first-line antituberculous agents or who are infected with drug-resistant organisms (Marra *et al.*, 2005).

The present study was aimed to evaluate the *in vitro* interaction of metformin hydrochloride with levofloxacin and its influence on protein binding.

Materials and Methods

Materials: Metformin hydrochloride (generously donated by United Chemicals & Pharmaceuticals Ltd, Chittagong, Bangladesh), levofloxacin (generously donated by Royal Pharmaceuticals Ltd, Chittagong, Bangladesh), bovine serum albumin (Fraction V, 96-98%, SIGMA-Aldrich), semi-permeable membrane (Medicinel, England), sodium-bi-carbonate (Merck, Germany), M/15 phosphate buffer, hydrochloric acid (Merck, Germany), potassium dihydrogen orthophosphate (Merck, Germany), disodium hydrogen orthophosphate (Merck, Germany), orthophosphoric acid (Merck, Germany), potassium hydroxide (Merck, Germany), sodium hydroxide (Merck, Germany) and demineralized water (collected from Albion Laboratories Limited, Chittagong, Bangladesh) were used.

Methods: The protein binding experiments were carried out according to the procedure of earlier studies by Amran *et al.* in 2008.

Preparation of bovine serum albumin (BSA) solution: 100 ml solution of 1×10^{-5} M was prepared by dissolving 0.3450 g of bovine serum albumin in M/15 phosphate buffer having pH 7.

Preparation of standard solutions: Metformin hydrochloride and levofloxacin were dissolved in demineralized water separately to produce their 1M solution. These stock solutions were diluted to desired strengths (1×10^{-5} M) by pH 7.4 buffer solution to get the working standard solution.

Preparation of standard curve: Standard curve was prepared to determine the drug concentration into the buffer compartment. Solution of different concentrations of metformin hydrochloride was prepared in M/15 phosphate buffer (pH 7.4) and the standard curve was constructed by plotting absorbance (measured at 232 nm) against concentrations (Figure 1).

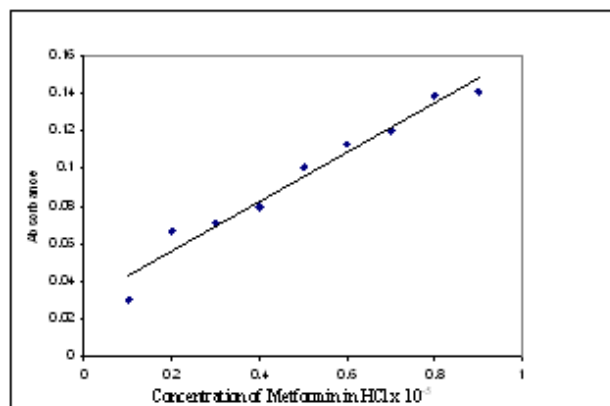


Figure 1. Standard curve of Metformin hydrochloride

Equilibrium dialysis method: Equilibrium dialysis is one of the methods used to determine protein binding of any compound. This method was used to determine the protein binding of metformin hydrochloride and its 1:1 mixture with levofloxacin (Nath *et al.*, 2010).

Dialysis procedure: The activated membranes were filled with 4 ml bovine serum albumin (BSA) solution with different concentrations of metformin hydrochloride or its 1:1 mixture with levofloxacin. Then, these were immersed in a 60 ml of M/15 phosphate buffer (pH 7.4) in 100 ml conical flask which was shaken gently at $37 \pm 0.5^\circ\text{C}$ for about 8 h. After completion of dialysis, the absorbance

of the buffer present outside of the membrane was measured at 232 nm.

Calculation of percentage of protein binding: Initially, a known amount of drug was taken into plasma compartment (dialysis bag). After equilibrium, concentration of drug present in the buffer of this compartment was measured. This measurement gave the total amount of drug that remains in the dialysis bag. Thus, it was possible to get sum of free drug and plasma bound drug at equilibrium. The percentage of protein bound drug was calculated by using the following formula.

The percentage of protein binding (F) is given by-

$$F = \frac{B - A}{B} \times 100$$

Here,

A = Molar concentration of free drug in buffer compartment

B = Molar concentration of total drug in protein compartment

Calculation of number of binding sites and the affinity constants: Number of binding sites and affinity constants of metformin hydrochloride and its 1:1 mixture with levofloxacin was calculated by using scatchard method (Singlas, 1987; Goldstein *et al.*, 1974; Scatchard, 1949).

Statistical analysis: The results were expressed as Mean \pm SEM values for each experiment. Differences in mean values between experimental groups were analyzed by unpaired t-test. A probability value less than 0.05 ($p < 0.05$) was defined to be significant.

Results and Discussion

Protein binding versus concentration of metformin hydrochloride indicated that at low concentration, the percentage of protein binding increased with the increase in concentration of the drug (Figure 2). But at higher

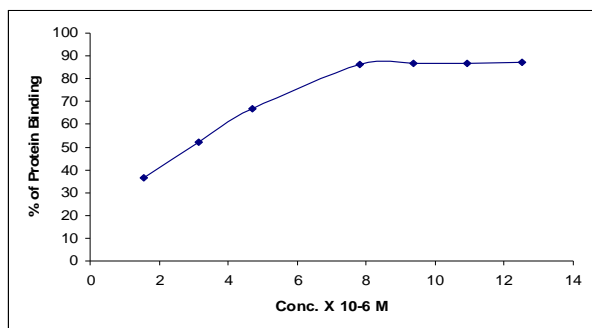


Figure 2. Protein binding of Metformin Hydrochloride alone

concentrations, the percentage attained a steady state indicating the saturation zone for the binding of metformin hydrochloride to bovine serum albumin (BSA). In the present study, the percentage of binding of metformin hydrochloride to BSA at saturation level was about 88%.

Highest percentage of protein binding with metformin hydrochloride at saturation level was about 90% in presence of levofloxacin (Figure 3). Interpreting Figure 2 and Figure 3, it can be resolved that levofloxacin has significant effect on the protein binding of metformin hydrochloride. This is due to an affinity of the complex and also levofloxacin for the protein.

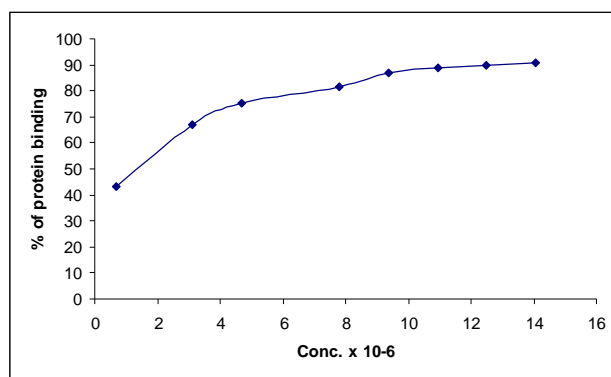


Figure 3. Protein binding of Metformin hydrochloride in presence of Levofloxacin (1:1 mixture)

According to Scatchard plots, the number of binding sites for metformin hydrochloride alone in BSA was found to be 0.026 and 2.090 for class I and II, respectively. The affinity constants k_1 and k_2 associated with class I and class II were 1.170 and 0.040, respectively (Figure 4).

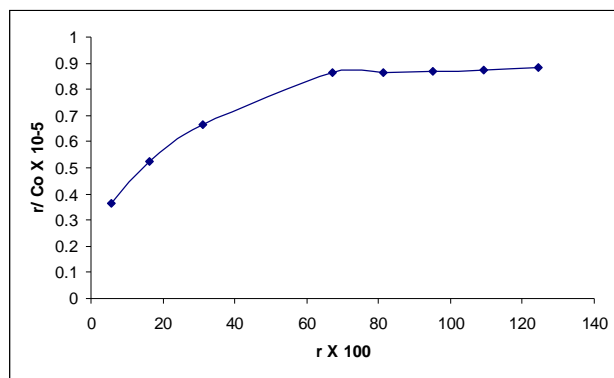


Figure 4. Scatchard plot for protein binding of Metformin Hydrochloride to BSA

The number of binding sites for Metformin-Levofloxacin system in BSA was found to be 0.346 and 0.702 for class I and II, respectively. The affinity constants k_1 and k_2 associated with class I and II were 0.112 and 0.110, respectively (Figure 5).

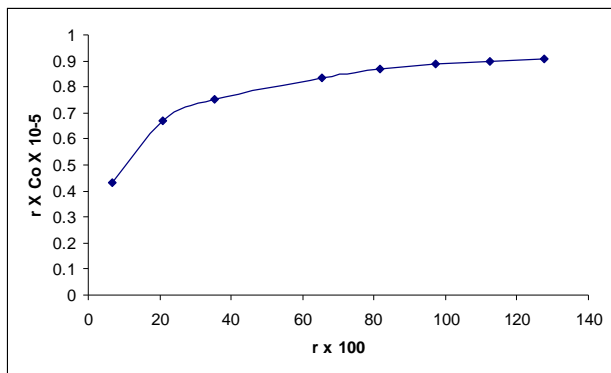


Figure 5. Scatchard plot for protein binding of Metformin hydrochloride in presence of Levofloxacin (1:1 mixture)

From the above table it can be concluded that in class I binding sites, a value of affinity constants for metformin hydrochloride is higher than its 1:1 complexes with levofloxacin, i.e. the presence of levofloxacin with metformin hydrochloride at physiological conditions, produced a decrease in values of affinity constant. Again in class II binding sites it was observed that values of affinity constants for metformin hydrochloride was higher than that of its 1:1 complexes with levofloxacin, i.e. the presence of levofloxacin with metformin hydrochloride showed an increase in values of affinity constant specially at lower concentration. In class II, number of binding site of metformin hydrochloride increases from 2.090 to 0.702 when mixed with levofloxacin. This is due to the decreased affinity for the drug to plasma protein binding. The volume of distribution of metformin hydrochloride may increase, because affinity of a drug for protein binding is a limiting factor of distribution of the drug.

Table 1. Number of binding sites and affinity constants

Systems	Class I			Class II		
	$n_1 k_1$	k_1	$n_{1x} 10^{-3}$	$n_2 k_2$	k_2	$n_{2x} 10^{-3}$
Metformin hydrochloride alone	0.309	1.170	0.026	0.836	0.040	2.090
Metformin hydrochloride + Levofloxacin	0.382	0.112	0.346	0.772	0.110	0.702

Conclusion

Due to decrease in affinity to plasma protein binding, there is an increase in the apparent volume of distribution (V_d) of the drug because, the affinity of a drug for protein binding is a limiting factor of the distribution of the drug (Hansten and Horn, 1989). In other words due to increase in affinity, the V_d decreased. Since, the apparent volume of distribution increases in the both cases it is a matter of concern that concurrent application of metformin hydrochloride and levofloxacin should be considered only after through *in vivo* studies.

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