Protein Binding of Glipizide Using Equilibrium Dialysis Technique: Effects of Hydrogen Ion Concentration, Drug Concentration and Ionic Strength

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The objective of this research was to investigate the effects of hydrogen ion concentration, drug concentration and ionic strength on the binding affinity of glipizide to albumin protein. Different buffer solutions of different pH values (pH 6.7, 7.5 and 8.5), different drug concentrations (2.45 mg, 4.82 mg and 9.42 mg), and phosphate buffer solutions pH 7.5 of different ionic strength (0.1, 0.4 and 1.0) were prepared. The effects of pH, drug concentration and ionic strength on the amount of glipizide bounded to 0.25 g bovine albumin was investigated. As the pH of the solution was increased from pH 6.4 to pH 8.5, milligrams drug bounded to gram protein (r value) decreased from 8.2 mg to 3.84 mg/g protein. Also as the ionic strength of the solution was increased from 0.1 to 1.0, the r value decreased from 10.76 mg to 3.96 mg/g protein. However, the r value did not change significantly with increasing of drug from 2.45 mg to 9.42 mg/25 ml. The r value was 7.36 mg/g protein when concentration of the drug was 2.45 mg/25 ml and 7.4 mg/g protein when the concentration of the drug was 9.42 mg/25 ml.

This study demonstrated that factors such as high pH and high ionic strength can alter drug-protein binding and consequently increase free drug in plasma and increase bioavailability of slightly water insoluble drug such as antidiabetic drugs.

Key words: Glipizide, Glipizide-protein binding, Ionic strength, Effect of pH on drug-protein binding, Equilibrium dialysis.

Drug binding to various blood and tissue proteins can influence the bioavailability and distribution of drugs. This is extremely important for drugs that exhibit low solubility in water portion of the plasma, since only free drug (unbound) is available for activity. Drug protein binding slows the disappearance of free drug from plasma into tissues by decreasing concentration gradient and also providing a source of free drug to replace that removed by various distribution and elimination process (1-3).

Methods for investigating drug protein binding can be summarized into two categories: a) equilibrium methods based upon changes in ligand concentration as a result of binding equilibrium and b) direct measurements method in which during the binding process characteristics of the drug protein or complex may be examined (4-9).

Judi et al. (10) studied the binding of tolbutamide, acetohexamide and chlorpropamide to serum protein using tromethamine buffer at three different pH using equilibrium dialysis method. They found that as the pH was increased, the binding sites value was decreased and that the association constants for chlorpropamide were approximately the same at all pH used. The affinity of the molecules for a protein is the summation of various factors and it is difficult to interpret the effect of pH on the association constant. A change in pH can affect the ionization of the molecules as well as can influence the number of binding sites available for binding. Hsu et al. (11) studied the interaction of acetohexamide, tolbutamide and chlorpropamide with plasma proteins by fluorescence method. Chlorpropamide showed the lowest binding affinity to bovine serum albumin.

No research has been found in the literatures related to glipizide protein binding. This is the first study that investigate the effects of factors such as pH, ionic strength and drug concentration on glipizide-protein binding. The association constants and the moles of drug bounded to moles of protein were determined.

Materials

Except when noted, all chemicals were analytical grade and used as received. Glipizide was generously supplied by Pfizer, Inc. (Puerto Rico) and bovine albumin protein (Sigma, U.S.A.).

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Methods

Determination of equilibrium time. A dialysis cell containing 50 ml of bovine albumin solution (10 mg/ml) was introduced in a beaker containing 50 ml of glipizide solution (0.2 mg/ml). The solution in the beaker was stirred in magnetic stirrer up to 24 hours. Samples of 5 ml were withdrawn at 0, 2, 4, 6, 8, 10, 12, and 24 hours and were replaced with 5 ml phosphat5e buffer solution. The absorbances at wave length of 276 nm were determined and drug concentration was calculated from the slopes and intercepts obtained from glipizide in phosphate buffer solution.

Effect of drug concentration on drug protein binding. Twenty five milliliters (25 ml) bovine albumin solution (10 mg bovine albumin/ml) and twenty five milliliters (25 ml) glipizide solution of different concentration (0.1 mg/ml, 0.23 mg/ml, and 0.4 mg/ml) were introduced in the dialysis sac. The dialysis sac was immersed in beaker containing 50 ml phosphate buffer pH 7.5. The solution in the beaker was stirred using magnetic stirrer for 24 hours. Samples of 5 ml were withdrawn after equilibrium at 24 hours. The amount of drug was determined by measuring the absorbances at a wave length of 276 nm using U.V. spectrophotometer.

Effect of hydrogen ion concentration on drug protein binding. Twenty five milliters (25 ml) bovine albumin solution (10 mg bovine albumin/ml) and 25 ml of glipizide solution (0.2 mg/ml) were introduced into dialysis sac. The dialysis sac was immersed in beaker containing 50 ml buffer solutions of different pH (pH 6.4, pH 7.5, and pH 8.5) in order to evaluate the effect of pH on drug binding affinity to bovine albumin. After equilibrium (24 hours) samples of 5 ml were withdrawn and the amount of drug was determined by measuring the absorbance at a wave length of 276 nm using U.V. spectrophotometer.

Effect of ionic strength on drug protein binding. Twenty five milliliters (25 ml) bovine albumin solution (10 mg/ml) and 25 ml glipizide solution (0.2 mg/ml) were introduced into dialysis sac. The dialysis sac was immersed into beaker containing 50 ml buffer solution of different ionic strength (0.1, 0.4, and 1.0) in order to evaluate the effect of ionic strength on glipizide protein binding. Samples of 5 ml were withdrawn after equilibrium 924 hours). The amount of drug in the sample was determined by measuring the absorbance at a wave length of 276 nm using U.V. spectrophotometer.

Results

Table 1 shows the free drug concentration remaining outside the dialysis sac (in the beaker) at different time intervals when buffer solutions of different pH were investigated. Figure 1 depicts the equilibrium time in buffer solutions of different hydrogen ion concentration. It is apparent that the equilibrium time of glipizide in different buffer solutions is approximately 24 hours.

Table 1. Free Drug Concentration Remaining Outside The Dialysis Sac

Time (hours)	Concentration of free drug outside the dialysis sac (in buffer solution of different pH)		
	PH 6.4	pH 7.5	pH 8.5
0	9.7	p.6	9.4
2	8.6	8.45	8.23
4	5.7	4.69	4.17
6	4.79	3.40	3.25
8	3.50	2.74	2.53
10	3.33	2.72	2.45
12	3.01	2.68	2.33
24	2.74	2.50	2.25

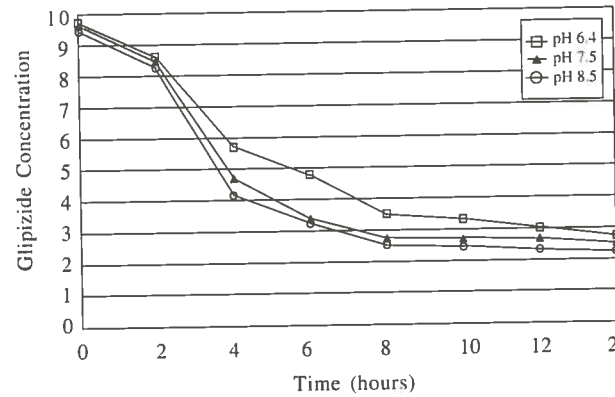

Figure 1. Equilibrium time of glipizide in buffer solution of different pH

Table 2 and Figure 2 show that the amount of free drug not bounded to protein increased as the ratio of drug to bovine albumin was increased. The milligram drug bounded per gram of bovine albumin (r value) appears to be constant (approximately 7.4 mg) and did not change as the drug concentration increased.

Table 2. Effect of Drug Concentration on Glipizide Protein Binding

Drug concentration	Drug bounded (mg)	Free drug (mg)	r (mg drug bounded/g protein)
2.45	1.84	0.74	7.36
4.82	1.86	2.96	7.44
9.42	1.85	7.72	7.40

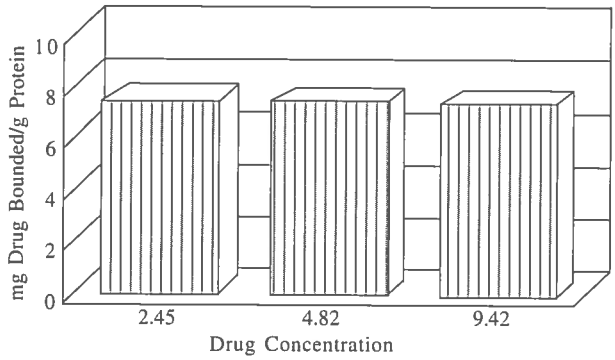

Figure 2. Effect of glipizide level on the degree of binding with protein.

Table 3 and Figure 3 depict the effect of pH of the buffer solution on the r value. As the pH of the buffer solution was increased, the r value was decreased. The r value was 8.2 mg glipizide per gram bovine albumin when the pH of the buffer solution was 6.4 while the r was 3.84 mg glipizide per gram of bovine albumin when the pH of the buffer solution was 8.5.

Table 3. Effect of the pH of Buffer Solution on Glipizide Protein Binding

pH of the buffer solution	Drug bounded (mg)	Free drug (mg)	r (mg drug bounded/g protein)
6.4	2.05	2.46	8.2
7.5	1.86	2.96	7.44
8.5	0.96	3.88	3.84

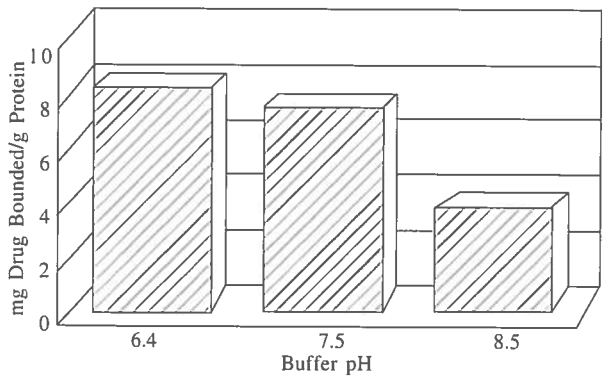

Figure 3. Effect of pH of buffer solution on amount of glipizide bounded per gram protein.

Table 4 and Figure 4 show the effect of ionic strength of the buffer solution on glipizide protein binding. The r value was 1.76 mg per gram bovine albumin when the ionic strength was 0.1 and was 3.96 mg per gram glipizide when the ionic strength was increased to 1.0. As the ionic strength of the buffer solution was increased, the amount of drug bounded per gram of bovine albumin was decreased.

Table 4. Effect of Ionic Strength on Glipizide Protein Binding

Ionic strength of buffer solution pH 7.5	Bounded drug (mg)	Free drug (mg)	r (mg drug bounded/g protein)
1.0	0.99	4.0	3.96
0.4	1.86	2.96	7.44
0.1	2.69	1.86	10.76

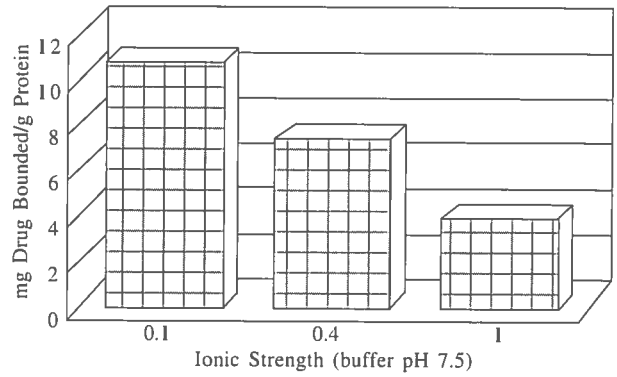

Figure 4. Effect of ionic strength of buffer pH 7.4 on the amount of glipizide bounded per gram protein.

Conclusions

This study demonstrated that factors such as drug concentration, hydrogen ion concentration and ionic strength had an effect on the amount of drug bounded to bovine albumin protein. Additionally, this study showed that as the drug concentration was increased, the binding site value decreased for glipizide and that the amount of drug bounded per gram bovine albumin was constant at all drug concentration used.

Factors such as high ionic strength and high hydrogen ion concentration can decrease drug protein binding and consequently can enable a sufficient concentration of free drug to develop at the receptor site and increase the bioavailability of water insoluble drug such as glipizide.

Resumen

El objetivo de este estudio fue investigar los efectos de la concentración del ion de hidrógeno, la concentración de la droga y la fuerza iónica en la afinidad de enlace de glipizide a la proteina de albumina. Se prepararon varias soluciones amortiguadoras con diferentes valores de pH (pH 6.7, 7.5 y 8.5), diferentes

concentraciones de droga (2.45 mg, 4.82 mg y 9.42 mg), y soluciones amortiguadoras de fosfato pH 7.5 de diferentes fuerzas iónicas (0.1, 0.4 y 1.0). Los efectos del pH, concentración de droga y fuerza iónica en la cantidad de glipizide enlazada a 0.25 g de albumina (bovine albumin) fue investigada. A medida que el pH de la solución fue aumentado de pH 6.4 a pH 8.5, los miligramos de droga enlazada a gramos de proteinas (valor R) disminuyeron de 8.2 mg a 3.84 mg/g proteina. Además, a medida que la fuerza iónica de la solución fue aumentada de 0.1 a 1.0, el valor r disminuyó de 10.76 mg a 3.96 mg / g proteina. Sin embargo, el valor r no cambió significativamente con el incremento de droga de 2.45 mg a 9.42 mg/25 ml. El valor r fue 7.36 mg /g proteina cuando la concentración de droga fue 2.45 mg / 2 ml y 7.4 mg / g proteina cuando la concentración de droga fue 9.42 mg / 25 ml. Este estudio demostró que factores tales como pH alto y fuerza iónica alta pueden alterar el enlace droga-proteina y por consiguiente aumenta la droga libre en plasma y aumenta la biodisponibilidad de drogas poco solubles en agua como las drogas antidiabéticas.

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