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In vitro and *in vivo* Effects of Glipizide and Gliclazide on the Protein Binding, Plasma Concentration and Serum Glucose, Cholesterol and Creatinine Levels of Ibuprofen

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Abstract: The *in vivo* and *in vitro* study of effects of glipizide and gliclazide on protein binding and plasma concentration of ibuprofen has been conducted by equilibrium dialysis method at physiological temperature (37±0.5)°C and pH (7.4) and the measurements have been done by UV-spectrophotometry. It has been found that the percentage of protein binding of ibuprofen alone was 91% and in 1:1 mixtures with glipizide and gliclazide were 80 and 82%, respectively, at the saturation levels. The binding sites for ibuprofen-gliclazide system were found to be 3.1 and 2.11 and the binding constants were 0.37 and 0.45, respectively. Both glipizide and gliclazide lowered the affinity and percentage of binding of ibuprofen to serum albumin. It has been found that the interaction of glipizide and gliclazide with ibuprofen increased the free drug concentration of ibuprofen in plasma. It has been found that plasma concentration of ibuprofen after oral administration with glipizide and gliclazide is lowered than in the case of ibuprofen alone. On the other hand, it has been found that co-administration of ibuprofen and glipizide reduces blood sugar slightly but gliclazide reduces significantly but the values of cholesterol and creatinine are not lowered in the cases of gliclazide and glipizide in presence of ibuprofen, rather they are seen to be higher. But the management of cholesterol and creatinine by gliclazide and glipizide are difficult tasks and leads to complications in many cases. It is thus clear that ibuprofen can be safely used in a combination therapy with gliclazide and better affectivity can be achieved.

Key words: Protein binding, ibuprofen, glipizide, gliclazide, binding sites, better effectively

INTRODUCTION

Plasma protein binding is one of the important and useful pharmacokinetics parameters of a drug. There are multiple binding sites on a protein molecule. The pharmacokinetics and pharmacodynamic behavior of a drug is governed by the strength of a complex formed by the drug molecules with the protein (Singlas, 1987). Drugs generally form reversible

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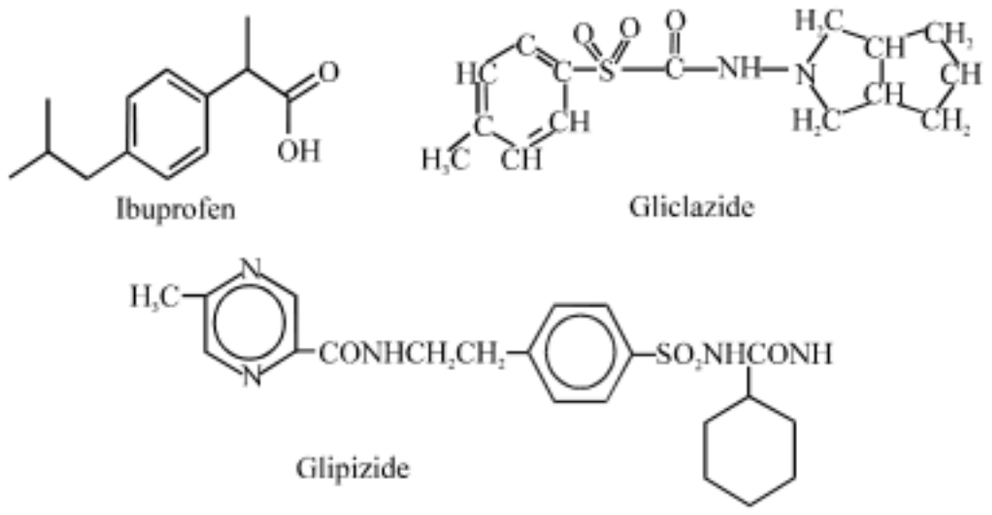

Fig. 1: Structures of ibuprofen, gliclazide and glipizide

complexes with the plasma proteins that act as a reservoir releasing the free drug to the circulation. The free form of the drug shows the pharmacologic response, is metabolized and excreted (Salam and Hossain, 2001). The bound drug is gradually released to maintain an equilibrium and thus the pharmacologic response is maintained. The type and nature of protein binding depend on the physicochemical properties of the drug molecule, its concentration, pH of the medium and also on the concentration and number of available binding sites of the plasma proteins (Mohiuddin *et al*., 2009a). Protein binding of a drug is a limiting factor for the drug effect (Hossain and Amran, 1999). Simultaneous administration of two or more drugs into the systemic circulation can modify the affinity of the drug to bind with plasma protein and thus percentage of protein binding. Due to this influence, a combined therapy can change the volume of distribution, renal and hepatic clearance and hence drug effects (Begum *et al*., 1988).

The concentration of drug in blood is easily accessible. The pharmacological response is influenced by the plasma concentration of drug. It is observed that there is an optimum or desired therapeutic or pharmacological concentration range over which the drug produces its characteristic effect (Mohiuddin *et al*., 2009b; Rahat *et al*., 1999). Drug-drug interaction result when one drug alters the known therapeutic response of another drug that has been administered concurrently. The result may be enhanced or diminished effects of one or both of the drugs. It is a common practice for the medical practitioners to prescribe more than one drugs in a single prescription and patients receive those drugs simultaneously. The effect of this simultaneous intake sometimes be neither safe nor effective but may be deleterious (Cadwallader, 1985).

The aim of the present study is to evaluate the effect of glipizide and gliclazide (Fig. 1) on the protein binding and plasma concentration of ibuprofen (Fig. 1) and thus to infer about the consequences of combined drug therapy for these drugs.

MATERIALS AND METHODS

Protein Binding Study

Equilibrium Dialysis

Protein binding was determined by the equilibrium dialysis method. For this purpose the membrane pieces (12 cm long and 3 mL capacity) were activated by digestion with 1.0 M sodium bicarbonate at 70°C for 4 h and washing thoroughly with deionized water thereby and immersing them in 0.067 M phosphate buffer of pH 7.4. Activated membrane bags were filled with solutions of serum bovine albumin with different concentration of the drugs and their

mixtures. The membrane bags were immersed in a fixed amount (50 mL) of phosphate buffer and the system was shaken gently for 6 h in a metabolic shaking incubator at 37±1°C. After this period the absorbance of the buffer samples (outside the membrane bags) were measured at 240 nm and the concentrations of the bound and unbound drugs were calculated with the help of the relationship shown in the next page using a standard curve (Singlas, 1987; Mohiuddin *et al*., 2009a).

Preparation of Standard Curve

A standard curve for ibuprofen was prepared using solutions of different concentrations of the drug in the buffer using a fixed concentration of either gliclazide or glipizide and measuring their absorbance at 223 nm which was found to be the $\ddot{e}_{max}$ of ibuprofen at lower concentrations (5-100 μM). The absorbance were plotted against the concentration of ibuprofen keeping the concentration of glipizide and gliclazide constant.

Calculation of Percentage of Protein Binding

The percentage of protein binding (F) is given by

$$F = \frac{[B]-[D]}{[B]} \times 100$$

Where:
[D] = Molar concentration of free drug in buffer compartment after dialysis
[B] = Molar concentration of total drug in protein compartment before dialysis

Calculation of Affinity Constants and Number of Binding Sites

The Scatchard method (1949) was used for this purpose and a curve was thus produced by plotting r/[D] versus r using the equation:

$$r = \frac{[B]-[D]}{[Protein]}$$

where, r is the ratio between the molar concentration of the bound drug and the molar concentration of protein. The curve so obtained when extrapolated gave an intercept on the Y axis representing nK, the intersection on the X-axis representing n and the slope of the line being K, where, n is the number of binding sites on the protein available to bind drug molecules or their complexes and K is affinity constant for the binding of a drug molecule or its complex.

Study of Plasma Concentration

Test Animals and Administration of Drugs

Plasma samples were taken at various time intervals after a drug was orally administrated. Sixteen adult rats of 250±25 g body weight were used. They were kept rest for 7 days with normal diet. These rats were divided into 4 groups each having 4, marked as 1, 2 and 3 and one group was used as control. Here, caffeine alone and its 1:1 mixture with gliclazide and metformin HCl were administrated by orogastric tube individually in each group. They were over night fasted before drug administration. Blood samples (1 mL) were collected from cutting the tip of the tail into centrifuge tubes before drug administration and at 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 h after drug administration. All blood samples were protected from light, immediately centrifuged at 3000 rev min^{-1} f or 10 min and the plasma samples were

separated into vials and kept into deep freeze until taking the absorbance. The absorbance was measured at 273 nm and the plasma concentration of caffeine was determined by using calibration curve (Ara *et al*., 2008; Mohiuddin *et al*., 2009b; Rahat *et al*., 1999).

Study of Blood Sugar, Cholesterol and Creatinine Level

A total of 42 rats were brought into diabetic type-2 status under the application of streptozocine, which is a potentially effective hyperglycemic agent and the required does was 90 μg kg^{-1}. For each combination of drugs such as ibuprofen-glibenclamide against glibenclamide alone, ibuprofen-glipizide against glipizide alone and ibuprofen-gliclazide against gliclazide alone. 12 rats (two groups, 6+6), aged about three months, each weighing 150-200 g were used for each drug combination. The six other rats were used as control. Blood samples (1 mL) were collected from cutting the tip of the tail into centrifuge tubes at fasting condition. All blood samples were protected from light, immediately centrifuged at 3000 rpm for 10 min and the plasma samples separated into vials and kept into freezer until taking the absorbance (Baki and Hossain, 1999).

Estimation of Blood Glucose Level

The blood glucose level was measured by the glucose oxidase method starting at the initial and after every three days of application of therapy in all experimental rats as well as for the control. Blood samples were collected from cutting the tip of the tail into centrifuge tubes and were centrifuged for 10 min. Five microliter of the standard/sample solution (1, 2, 4, 8, 12, 16, 20 mmol L^{-1}) were kept in different well of the microfiltration plate. Two hundred and fifty microliter reagent (oxidase enzyme) was added to the solution of the each well and the plate was kept in a incubator for 18 min at 37°C. Absorbance at 490 nm was taken and concentration of glucose was determined (Baki and Hossain, 1999).

Estimation of Blood Cholesterol Level

The blood cholesterol level was measured by enzymatic method. Blood samples were collected and prepared for experiments in an analogous method and absorbance at 500 nm was taken and the concentration of cholesterol was determined (Baki and Hossain, 1999).

Estimation of Blood Creatinine Level

The blood creatinine level was measured by enzymatic method. Blood samples were collected and prepared for experiments in an analogous method and absorbance at 510 nm was taken and the concentration of creatinine was determined (Baki and Hossain, 1999).

RESULTS AND DISCUSSION

The percentage of protein binding of ibuprofen was 91% and in 1:1 mixtures with glipizide and gliclazide were 80 and 82%, respectively, at saturation level (Fig. 2). It is seen that the percentage of protein binding of ibuprofen is markedly decreased in presence of glipizide and gliclazide, though the extents become almost similar at high concentrations of the drugs. It is known that the more is the protein binding of a drug the less will be its therapeutic effect.

The number of binding sites for ibuprofen alone were 0.75 and 1.37 for class I and class II binding sites and the binding constants were 16 and 4.8, respectively. The number

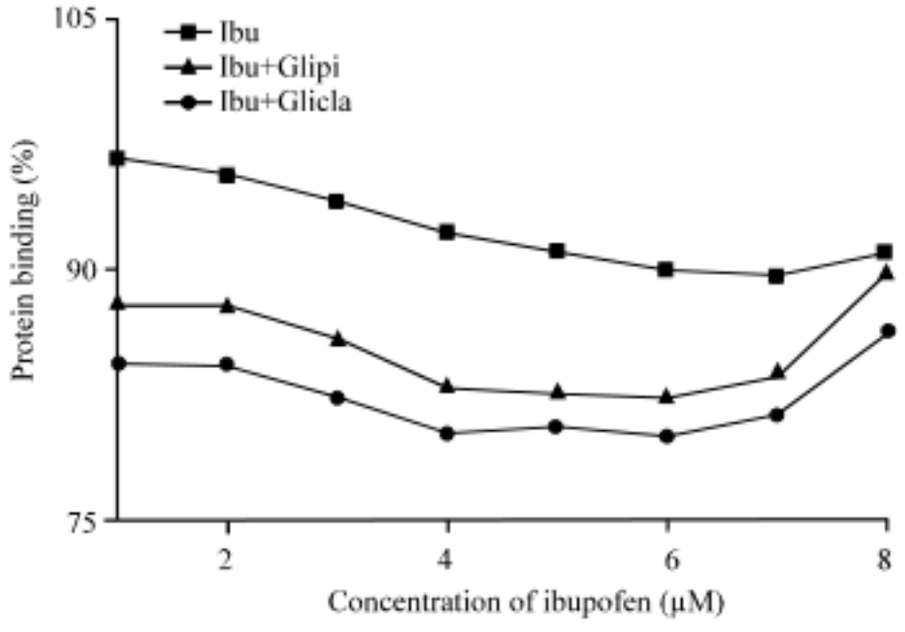

Fig. 2: Protein binding of ibuprofen in presence of glipizide and gliclazide

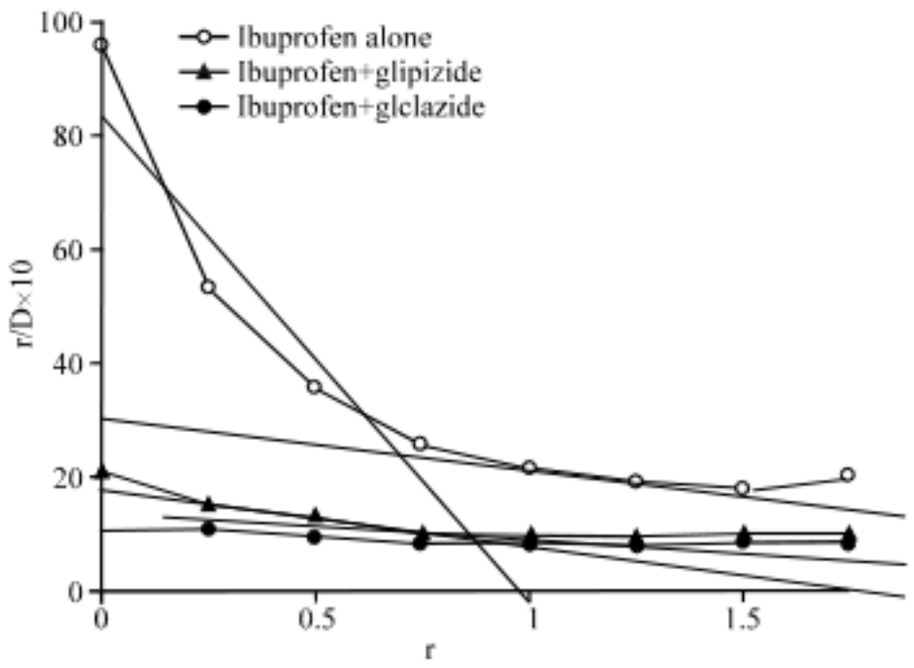

Fig. 3: Scatchard plot for protein binding of ibuprofen and its 1:1 mixtures with glipizide and gliclazide

Table 1: The values for number of binding sites and affinity constants

	Class I binding sites			Class II binding sites		
Systems	n_1K_1	K_1	n_1	n_2K_2	K_2	n_2
Ibuprofen	12.00	16.00	0.75	4.80	3.50	1.37
Ibuprofen+glipizide	2.35	2.30	1.01	1.05	0.17	6.17
Ibuprofen+gliclazide	1.15	0.37	3.10	0.95	0.45	2.11

of binding sites for the ibuprofen-glipizide systems were found to be 1.01 and 6.17 and the binding constant being 2.32 and 0.17, respectively. The binding sites for ibuprofen-gliclazide systems were found to be 3.1 and 2.11 and the binding constants were 0.37 and 0.45, respectively (Fig. 3, Table 1). Since, the value of n_1k_1 and n_2k_2 are 12 and 4.8, respectively, the lone drug (ibuprofen) has large affinities for serum protein binding and the interacting drug molecules (glipizide and gliclazide lower these affinities (values being 2.35, 1.15, 1.05 and 0.95, respectively)). These two drugs compete with ibuprofen sufficiently to occupy the binding sites in all the conditions studied.

Effects of Glipizide and Gliclazide on the Plasma Concentration of Ibuprofen

The pharmacological response is influenced by the plasma concentration of drug. The plasma concentration of ibuprofen alone was 95 ng mL^{-1} and in the case of ibuprofen-glipizide it has been found to be 102 ng mL^{-1} while, in the case of ibuprofen-gliclazide the value was 115 ng mL^{-1} (Fig. 4). Therefore, the enhancement of the values of ibuprofen in presence of glipizide and gliclazide is supportive of the values of protein binding of ibuprofen in presence of these molecules (Mohiuddin *et al.*, 2009b).

Influence of Ibuprofen on Hypoglycemic Properties of Glipizide and Gliclazide

The affect of ibuprofen on hypoglycemic effects of glipizide and gliclazide were measured on rats after inducing diabetes in then by administration of streptozotocine keeping a control group only on normal diet. The values of serum glucose, cholesterol and creatinine were measured initially, after 3 days, 6 days (data not shown in the Table 2) and after 9 days of drug administration (Table 2).

It is seen that ibuprofen has good effects on antiglycemic activities of gliclazide and glipizide in streptozotocine-induced diabetic rats. However, the management of cholesterol and creatinine by gliclazide and glipizide are difficult tasks and leads to complications in many cases.

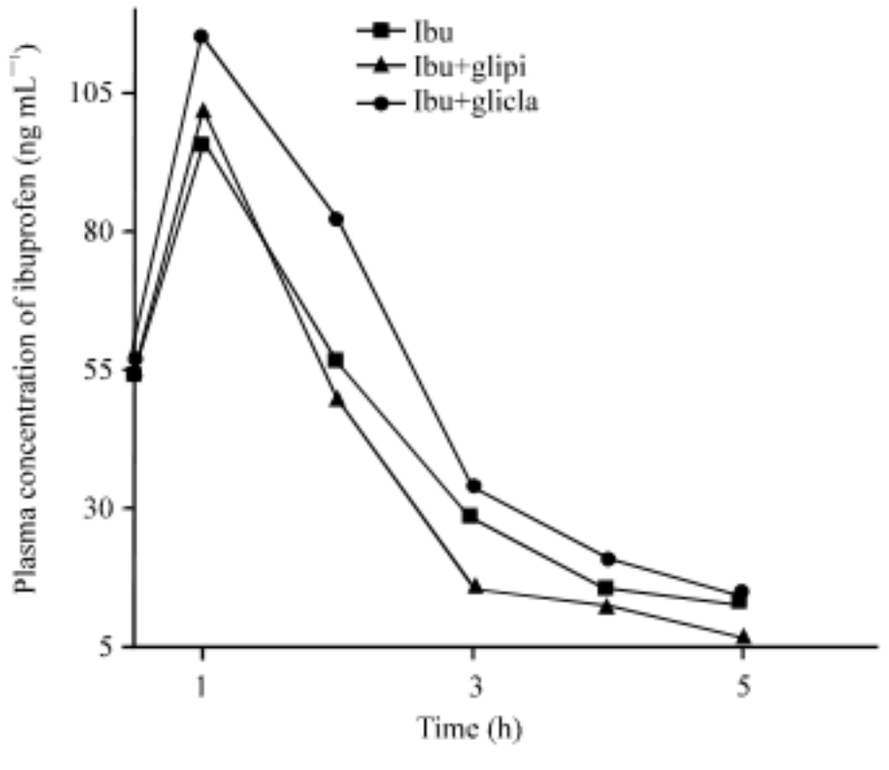

Fig. 4: Plasma concentration of Ibuprofen after oral administration of Ibuprofen alone and its mixture with glipizide and gliclazide in rats

Table 2: Serum glucose (mmol L^{-1}), cholesterol (mg dL^{-1}) and creatinine (mg dL^{-1}) levels of the controls and streptozotocine-induced diabetic rats at initial and after 9 days of drug administration in the cases of antidiabetic molecules and their mixtures with ibuprofen

	Control		Gliclazide		Glipizide		Glicla+Ibu		Glipi+Ibu	
Parameters	Initial	After 9 days	Initial	After 9 days	Initial	After 9 days	Initial	After 9 days	Initial	After 9 days
Glucose	6.50±1.4	9.3±1.4	8.2±1.7	6.8±0.8	9.2±1.6	6.3±0.5	8.3±1.0	6.1±1.4	8.5±1.6	6.1±1.0
Cholesterol	74.70±8.5	84.2±19.2	78.2±14.6	65.5±10	61.8±8.6	57.2±13	81.2±9.8	73.8±18	90.0±4.8	65.7±8.9
Creatinine	0.80±0.3	1.2±1.6	0.8±0.2	0.7±0.2	1.0±0.2	0.7±0.1	1.1±0.3	0.7±0.2	0.9±0.2	0.7±0.1

The comparison of this study with our previous drug-drug interaction studies (Mohiuddin, 2009a-c; Amran *et al*., 2006, 2008) infer that the results of those studies do not differ significantly as shown in the current study as well.

CONCLUSION

Polypharmacy is a common practice in cases of patients undergoing a major operation, hospitalized patients and also in geriatric patients. We conclude on the basis of protein binding data and serum levels of ibuprofen in presence of glipizide and gliclazide that co-administration of ibuprofen with glipizide and gliclazide might be safe and effective.

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