USE OF IN VIVO ANIMAL MODELS TO ASSESS THE EFFECT OF GLIPIZIDE ON PHARMACOKINETIC AND ANTICONVULSANT ACTIVITY OF DIVALPROEX SODIUM

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ABSTRACT

The study was conducted to find the influence of Glipizide on the pharmacokinetic and anticonvulsant activity of Divalproex Sodium (DS). Both epilepsy and diabetes are managed clinically by administering various drugs for prolonged period of time. Therefore, polypharmacy are key factors, alarming drug-drug interaction. Healthy albino rabbits were used to study the effect of Glipizide (3.73 mg/kg p.o) on pharmacokinetic parameters of DS (25mg/kg p.o) followed by anticonvulsant activity to confirm the results. The experiment consists of two parts i.e administration of Divalproex alone and in combination with Glipizide after seven days treatment of Glipizide in four healthy albino rabbits. Blood samples were collected at 0, 30 min, 1st, 2nd, 4th, 8th and 16th hour from the marginal ear vein puncture of each rabbit. Serum concentrations were analyzed by a validated Ultra Performance Liquid Chromatography (UPLC) method. For pharmacodynamic study, electrically and chemically induced convulsion tests were used. The concentration of serum DS was found significantly increased after the Glipizide treatment at 1st, 4th, 8th and 16th hour for one week but it failed to exhibit the significant changes at 4th hour. The pharmacokinetic parameters like Area Under Curve (AUC), Area Under first order Moment Curve (AUMC), t1/2, Peak Plasma Concentration (Cmax), Mean Residential Time (MRT) and Time of maximum concentration (Tmax) of DS showed changes after Glipizide treatment for one week in healthy albino rabbits. Glipizide treatment for one week exhibited significant increase of duration of hind limb extensor time by maximal electroshock test and delayed onset of clonic convulsion in Pentylentetrazole induced seizures test. The results revealed that the drug-drug interaction between DS and Glipizide is at distribution and metabolism.
Keywords: Diabetes, divalproex sodium, drug interactions, Glipizide, pharmacokinetic parameters

INTRODUCTION
Epilepsy, the second most common chronic neurological condition seen by neurologist after stroke is characterized by recurrent seizures, which are caused by abnormal, rapid, synchronized neuronal discharges [1-3]. The estimated number of person with epilepsy in India is approximately 5.5 million, among whom approximately 4.1 million reside in rural areas [3]. Similarly, diabetes is another condition which affects large population in the world. A study conducted by Gaitatzis et al [4] showed that nine per cent of older people with epilepsy had diabetic in the UK. Danaei [5] reported that 347 million people worldwide have diabetes. Around 50 million people worldwide have epilepsy and nearly 80% of the people with epilepsy are found in developing regions [6].

Studies have been focused on the connections between numerous neurological disorders with non-neurological chronic illness. The two drugs selected for the present study are Divalproex Sodium (DS) and Glipizide used in treatment of neurological disorder like epilepsy with non-neurological chronic illness such as diabetes respectively. According to Tiamkao et al [7] epilepsy and diabetes have some common features, main commonality being fluctuating blood sugar. The study further showed that hyperglycemic people tend to have focal or local seizures. Another study by Fisher and Frier [8] reported that hypoglycemic people, are likely to have tonic-clonic seizures.

Both epilepsy and diabetes are managed clinically by administering numbers of drugs for long duration. The drugs DS and Glipizide are used in convulsion and diabetes respectively. DS or simply divalproex is a stable and unique preparation consisting of sodium valpraote and Valproic acid in a 1:1 molar relationship. It is formed during partial neutralization of Valproic acid with 0.5 equivalent of Sodium hydroxide. Glipizide, a second-generation sulfonylurea, is an oral hypoglycemic agent for the management of type II Diabetes Mellitus.

Whenever two or more drugs are being taken the action of one drug may affect the activity, metabolism, or toxicity of another drug. This is called Drug-Drug Interaction (DDI) or simply Drug Interaction (DI). The result may be enhanced or diminished effects of one or both of the drugs or the appearance of a new effect which is not seen with either drug alone [9]. In addition, patient’s response to therapy can be changed by DDI. Therefore, polypharmacy are
key factors, alarming drug-drug interaction which are important cause of adverse drug reaction and possibly increases health care cost along with chances of raised in hospitalization. Hence, there is a chance of one drug altering the effects of another. 

Since there is possibility for the combined use of Glipizide and DS in chronic diabetics associated with epilepsy, the present study has been taken up to evaluate the effect of Glipizide on pharmacokinetic and anticonvulsant activity of DS and also to predict the mechanism of interaction between them.

**MATERIALS AND METHOD**

**Animals**

Healthy adult albino rabbits (2.0 to 2.5kg), healthy albino rats (180 to 220g) and albino mice (20 to 25g) respectively were selected and obtained from the animal house of Mallige College of Pharmacy, Bangalore. Animals were housed in stainless steel cage and were provided with distilled water *ad libitum* throughout the experiment. All these animals were given standard pellet diet and were maintained under laboratory conditions [temperature (25±30C) and 12:12 hour natural light: dark cycle], humidity (75%) as per Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines. The animals were acclimatized to standard laboratory conditions before performing an experiment. The animal experiments were performed after approval from Institutional Animal Ethical Committee (IAEC).

**Chemicals**

Pure Glipizide was obtained as a gift sample from Micro labs, Hosur, Tamilnadu; Divalproex Sodium and Pentylenetetrazole (PTZ) was procured from Yarrow chemicals, Mumbai; Acetonitrile, High-Performance Liquid Chromatography (HPLC) and anti coagulant powder was purchased from S.D fine chemicals, Mumbai. All the chemicals used were of analytical grade.

**Apparatus**

The apparatus used in study were: Ultra Performance Liquid Chromatography (UPLC) [Aquity System]; Laboratory Centrifuge Remi R8C [Remi Motors, Mumbai]; Electronic balance [Adventurer balances, USA]; Petri dishes; Rabbit holder; Disposable syringe (1-3ml); 26 Gauge needles; Spatula; Sterilized cotton; Variable micropipette [0-50 µL]; Glass rod; Conical flask; Measuring cylinder [10-50 ml]; Refrigerator; Centrifuge tube; Epindrofs tube; Autoclave; and Oral cannula (18, 20 and 22gauze).
Methods

Preparation of Standard Glipizide, DS, and PTZ solution
Pure sample of Glipizide (accurately weighed 100mg) and DS (weighed 100mg) was dissolved with 0.5 ml of 0.1 N NaOH and 0.9% NaCl respectively. Final volume was adjusted with distilled water to get 10mg/ml of Glipizide and 20mg/ml DS stock solution respectively. Similarly, PTZ (accurately weighed 100 mg) was dissolved in distilled water and final volume was adjusted to get 10mg/ml stock solution.

Collection of blood sample
Blood samples were collected at 0, 30 min, 1st, 2nd, 4th, 8th and 16th hour from the marginal ear vein puncture of each rabbit. Serum was used for the estimation of DS concentration by using UPLC technique.

Chromatographic analysis of serum DV
1 ml serum sample was precipitated by adding 3 ml of Acetonitrile followed by centrifugation at 10000 r.p.m for 20 min. The aqueous/organic layer which was transported to a Silanized glass tube was evaporated under nitrogen and reconstituted with Acetonitrile. Reconstituted solution was filtered using membrane [Nylon-65] syringe filter.

The reconstituted samples after filtration were analyzed by newly developed and validated Aquity UPLC instrument with a double diode PDA detector set at 254 nm. Waters aquity software was used for data handling. The pre-column used was Vanguard 3/PK, 2.1×5mm column. The column used was an Aquity UPLC® BEH C18 2.1x100 mm, 1.7µm. The 2 µl of the serum was injected into the column. The two solvent system used were, solvent A (0.01% Formic acid in water) and solvent B (75% Acetonitrile in water). Flow rate was maintained at 0.400ml/min. The chromatogram was recorded and used for quantification.

Experimental Study design
Clinically both the drugs will be administered orally as antidiabetic and antiepileptic therapy. Human oral therapeutic doses of the respective drugs were extrapolated to mice/rat/rabbit based on body surface area [10].

The study consists of two parts.
Part-1: Effect of Glipizide treatment on the pharmacokinetic parameters of DS in healthy albino rabbits
Part-2: Effect of Glipizide treatment on anti-convulsant activity of DS
Part-1 Effect of Glipizide treatment on the pharmacokinetic parameters of DS in healthy albino rabbits

Overnight fasted male albino rabbits (n=4), weighing between 2.0 to 2.5 kg were selected. Experiment was divided into two phases. In first phase of experiment, animals were administered with solution of DS (25 mg/kg, p.o.). The time of drug administration was noted for all the animals. The blood sample was collected at 0, 30 min, 1st, 2nd, 4th, 8th and 16th hour after the DS administration. Serum was used for the estimation of serum DS concentration by using UPLC technique.

Two weeks washout period was maintained between treatments and then, same group of animals was maintained with daily treatment of Glipizide for a week. On the 7th day, 6 hours after administration of the drug, the rabbits were fasted for 18 hours. On the 8th day, DS was administered to the rabbits. Blood samples were collected for estimation of DS. The blood concentration of DS before and after Glipizide treatment were applied to software Ramkin to calculate pharmacokinetic parameters like AUC0-t, AUMC0-t, Cmax, Tmax, t1/2 and MRT.

Part-2: Effect of Glipizide treatment on anti-convulsant activity of DS was studied using two animal models

I. Electrical method [maximum electroshock (MES) induced convulsion]
II. Chemical induced method [Pentylenetetrazole (PTZ) induced convulsion method]

I. MES induced convulsion
Albino rats were divided into two groups each containing six animals. Group I received saline orally and served as a control while group II received 92.5 mg/ 0.15kg of DS orally. The DS and saline were given orally one hour prior to induction of convulsions. Electrically, seizures were induced in albino rats by using electroconvulsimeter, set to 150mA for 0.2 sec using ear clip electrodes after an hour of treatment [11]. The animals were observed for the extensor phase as well as its duration. A criteria for anticonvulsant activity was abolition of extensor (tonic) phase in drug treated group.

After a wash out period of two weeks the same groups of animals were administered with Glipizide once a day for one week. On the 8th day, DS and Glipizide were administered. The test was repeated and the duration of extensor phase was measured.
**II. PTZ induced convulsion method**

The albino mice weighing 20±5 g were selected prior to conducting the experiment by injecting the PTZ in a dose of 30 mg/kg body wt. subcutaneously in the scruff of neck [12]. Mice showing clonic convulsions (within 30 minutes) throughout initial study were selected. They were brought to the laboratory one day prior to the experiment and were housed separately in cages with free access to food and water.

Albino mice were divided into two groups each containing six animals. Group I which received PTZ (80mg /kg, i.p.), served as a control while group II received DS (185mg/0.02kg p.o.). After one hour of drug treatment, PTZ (80 mg/kg, i.p.) was given and then animals were observed for clonic convulsions in 30 minutes, duration of convulsions and 24 hour mortality [12-13]. A criteria for anticonvulsant activity was absence of clonic convulsions in drug treated group.

After a wash out period of two weeks the same animals were administered with Glipizide once daily for a week. On the 8th day, DS and Glipizide were administered. The test was repeated and duration of convulsions and 24 hour mortality was noted.

**Statistical Evaluation**

Pharmacokinetic data of DS was measured assuming complete oral absorption. All the experimental results were expressed as mean ± SEM and assessed by Students paired ‘t’ test using parametric statistics and GraphPad Prism Software, Inc; Version 6. A value of P <0.05 were considered statistically significant.

**RESULTS**

**Effect of Glipizide treatment on the pharmacokinetic parameters of DS in healthy albino rabbits**

DS is well absorbed after oral administration, concentration of 71.53 mcg/ml was observed at 4th hour and it is the peak effect (Table 1). It started declining at 8th hour i.e., 63.25 mcg/ml, and showed a sustained concentration of 58.32 mcg/ml at 16th hour.

Table 2 shows the mean pharmacokinetic parameters of DS administered alone and in combination with Glipizide as well as the statistical significance. DS pharmacokinetic parameters revealed that its peak effect is at 4th hour and t1/2 is around 9 hours. There is no significant change in Tmax of DS administered alone and in combination with Glipizide i.e at
4 hr indicating no change in rate of absorption. The effect on metabolism is significant since there is slightly increase in T1/2 and Cmax after Glipizide treatment.

There is also slight increase of terminal half-life from 9.07 to 11.47 hours. The peak concentration of DS changes significantly due to Glipizide treatment. Glipizide treatment showed no significant change on mean residence time of DS.

**Effect of Glipizide treatment on anti-convulsant activity of DS was studied using two animal models**

**MES induced method**

The duration of Hind Limb Tonic Extensor (HLTE) in rats treated with DS alone was 13.75±0.45 seconds and those treated with DS and Glipizide was 10.53±0.51 (Table 3). Glipizide treatment for one week alters the HLTE duration in healthy albino rats. The duration time is slightly reduced from 13.75±0.45 to 10.53±0.51 seconds. Incidence of convulsion in DS administered rat was 46% followed by 35% in DS and Glipizide treated.

**Table 1 Serum concentration of DS before and after Glipizide treatment in healthy albino rabbits (N=4)**

<table>
<thead>
<tr>
<th>Time (in hrs)</th>
<th>Serum concentration of Drug in mcg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS (Mean ± SEM)</td>
</tr>
<tr>
<td></td>
<td>DS + Glipizide (Mean ± SEM)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>33.25 ± 1.21</td>
</tr>
<tr>
<td>1</td>
<td>49.94 ± 1.73</td>
</tr>
<tr>
<td>2</td>
<td>55.97 ± 2.06</td>
</tr>
<tr>
<td>4</td>
<td>71.53 ± 2.41</td>
</tr>
<tr>
<td>8</td>
<td>63.25 ± 1.88</td>
</tr>
<tr>
<td>16</td>
<td>52.13 ± 1.36</td>
</tr>
<tr>
<td></td>
<td>40.26 ± 2.0**</td>
</tr>
<tr>
<td></td>
<td>55.75 ± 1.77**</td>
</tr>
<tr>
<td></td>
<td>62.63 ± 2.13**</td>
</tr>
<tr>
<td></td>
<td>78.56 ± 0.08**</td>
</tr>
<tr>
<td></td>
<td>67.26 ± 0.54*</td>
</tr>
<tr>
<td></td>
<td>58.32 ± 0.32**</td>
</tr>
<tr>
<td></td>
<td>*p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>**p&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 2 Effect of Glipizide treatment on pharmacokinetic parameters of DS in healthy albino rabbits (N=4)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DS (Mean ± SEM)</th>
<th>DS+ Glipizide (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC 0-t (mcg/ml/h)</td>
<td>940.64 ±3.88</td>
<td>1020.80 ±3.56</td>
</tr>
<tr>
<td>AUMC0-t (mcg/ml/h)</td>
<td>11008.69 ± 2.37</td>
<td>12080.08 ± 4.27</td>
</tr>
<tr>
<td>T1/2 (hrs)</td>
<td>9.07 ± 1.12</td>
<td>11.47 ±1.32</td>
</tr>
<tr>
<td>Cmax (mcg/ml)</td>
<td>71.41 ± 1.51</td>
<td>78.56 ±0.66</td>
</tr>
<tr>
<td>Tmax (hrs)</td>
<td>4 ± 0.06</td>
<td>4 ±0.36</td>
</tr>
<tr>
<td>MRT</td>
<td>11.71 ± 2.17</td>
<td>11.73 ±3.15</td>
</tr>
</tbody>
</table>

AUC- Area under curve
AUMC- Area under first order moment curve  
Cmax- Peak Plasma Concentration  
MRT- Mean residence time  
Tmax- Time of maximum concentration  
Tt1/2 – Terminal half life

**PTZ induced convulsion method**

The mice administered with DS (185 mg/0.02kg) alone exhibits latency period of clonic convulsion of 314.30 ± 0.71 seconds (Table 4). Percentage prolongation of clonic convulsion was enhanced to 336.07%. Glipizide treatment for one week increases the latency period of clonic convulsion in healthy albino rats significantly from 314.30 ± 0.71 seconds to 324.18±1.36. The nature of convulsion was jerky movements with lack of straub’s tail. Percentage prolongation of clonic convulsion was enhanced to 346.64%.

**Table 3** Effects of Glipizide treatment on anti-convulsant activity of DS on Maximal Electroshock (MES) induced convulsion in rats (N=6)

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Duration of Extensor tonus (sec) (Mean ± SEM)</th>
<th>Death/recovery</th>
<th>% Incidence of convulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline 5ml/kg)</td>
<td>29.35 ± 0.69</td>
<td>Recovery</td>
<td>100%</td>
</tr>
<tr>
<td>DS (92.5 mg/0.15)</td>
<td>13.75 ± 0.45</td>
<td>Recovery</td>
<td>46%</td>
</tr>
<tr>
<td>DS (92.5 mg/0.15) + Glipizide (10mg/kg)</td>
<td>10.53 ± 0.51</td>
<td>Recovery</td>
<td>35%</td>
</tr>
</tbody>
</table>

**Table 4** Effect of Glipizide treatment on anticonvulsant activity of DS by Pentylenetetrazole (PTZ) induced convulsion (N=6)

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Onset of clonic convulsion (sec) (Mean ± SEM)</th>
<th>Nature and severity</th>
<th>Death/recovery</th>
<th>% prolongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTZ (80mg/kg, i.p.)</td>
<td>93.52 ± 0.82</td>
<td>Straub’s tail, continuous jerky movements</td>
<td>Death</td>
<td>_</td>
</tr>
<tr>
<td>DS (185 mg/0.02 kg, p.o.) + PTZ (80mg/kg, i.p.)</td>
<td>314.30 ± 0.71</td>
<td>Less Jerky movements</td>
<td>Recovery</td>
<td>336.07%</td>
</tr>
<tr>
<td>Glipizide (10 mg/kg, p.o.) + DS(185 mg/0.02 kg, p.o.) sodium + PTZ</td>
<td>324.18±1.36</td>
<td>Jerky movements</td>
<td>Recovery</td>
<td>346.64%</td>
</tr>
</tbody>
</table>
DISCUSSION

Drug interactions are commonly seen in medical settings and its mechanism is studied in animal model (rodents and non rodents) [14-15]. We studied the influence of Glipizide on the pharmacokinetic and anticonvulsant activity of DS at therapeutic doses in healthy rabbits, rats and mice. Glipizide, second generation sulphonylurea is well known oral antidiabetic agent by pancreatic (stimulating insulin secretion by blocking K⁺ channel in the pancreatic β cells) and extra pancreatic (increasing tissue uptake of glucose) metabolism [14]. Glipizide slightly increase the anticonvulsant activity of DS in multiple dosages in healthy rabbits.

Single dosage of DS exhibits maximum concentration of 71.53±2.41. The pharmacokinetic parameters like AUC, AUMC and MRT were 940.64µg/ml/hr, 11008.69 µg/ml/hr and 11.71 hr respectively. But on multiple dosages of Glipizide the plasma levels and pharmacokinetic were slightly increased. In multiple doses of Glipizide studies, the results showed that co-administration of Glipizide increased the AUC of DS by 4.88%. These results suggest the increased plasma concentration of DS in Glipizide treated rabbits would be caused by increased in DS bioavailability.

The possible mechanism behind this type of interactions at pharmacokinetic level may be due to displacement of DS from protein binding. The enzymatic site for both DS and Glipizide is plasma protein-albumin. Glipizide and DS are substrate for CYP2C9 and later inhibits CYP2C9 as well as metabolized by CYP2C9 [9-16]. Most of the drugs widely used in clinical psychopharmacology are heptatically eliminated and circulate in the blood bound to plasma proteins. Often, the degree of binding is more than 90%. The assumption that protein-binding displacement interactions are generally of minimal clinical significance is not based on evidence, but rather the lack of it [17].

Glipizide is displacer drug and DS is displaced drug. After a week administration of Glipizide, the substrate concentration increased by increasing substrates concentration, the rate of reaction will increased due to the likelihood that number of enzymes-substrates complex will increase. This occurs until the enzyme concentration becomes limiting factor. Administration of Glipizide to rabbit on DS may results in displacement of later from its binding site. Displacement interactions can results in rise in free concentration of the displaced drug which may enhance pharmacodynamic response of Divalproex. However, our results are to be confined in healthy volunteer and convulsive patients to confirm the drug-drug interaction.
In future, research work can be continued into the metabolic process that lead to many of the clinically relevant drug interaction and role of genetic polymorphism of the drug metabolizing enzymes in determining how individuals respond to certain drug combinations. Glipizide treatment has slightly enhanced the anticonvulsant activity of DS in healthy rabbits. The interaction observed appears to be pharmacokinetic interaction at distribution and metabolic level and also at pharmacodynamic level. Hence, it is suggested the duration and frequency of DS has to be readjusted when both drugs are used together.

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