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PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTION STUDY OF CURCUMIN WITH REPAGLINIDE IN NORMAL AND DIABETIC RATS

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ABSTRACT

Aim: When herbal drugs or phytochemicals are taken along with conventional drugs, they modify the pharmacokinetics and pharmacodynamics of those drugs. Some reports proved that curcumin is a CYP3A4 inhibitor, so it is necessary to evaluate the interaction of curcumin with repaglinide. **Methods and Results:** The objective of the present study was to examine the influence of curcumin on the pharmacokinetics and pharmacodynamics of repaglinide in normal and streptozotocin (STZ) induced diabetic albino wistar rats. All the rats were divided into eight groups (n=6). First four groups were taken as normal rats and remaining four groups as STZ induced diabetic rats. Groups I & V animals treated with repaglinide, groups II & VI animals treated with curcumin, groups III, IV & VII, VIII animals treated with both curcumin and repaglinide for single dose and multiple dose interaction studies. Diabetes was induced in the animals intraperitoneally using 55 mg/kg streptozotocin. At particular time intervals blood samples were collected from rats via retro orbital plexus by using heparinised capillary tubes and pharmacokinetic and pharmacodynamic parameters were measured. Combination of repaglinide and curcumin significantly increased the Cmax, AUC and AUMC in both normal and diabetic rats and significantly decreased the clearance and Vd. Antihyperglycemic effect was significantly increased in multiple dose curcumin treated diabetic rats than single dose curcumin treated and repaglinide alone. **Conclusion:** Combination of curcumin and repaglinide significantly reduced the metabolism of repaglinide and it has more beneficial effect in diabetes mellitus. Repaglinide dose may be adjusted to prevent the complications.

INTRODUCTION:

Diabetes mellitus (DM) is one of the oldest diseases[1] and it is a chronic metabolic disorder characterized by hyperglycemia caused by insulin deficiency and/or insulin resistance.[1,2] The organs affected mainly in diabetes are liver and kidney.[1] Hence diabetes is one of the most prevalent and chronic disease which significantly reduces life expectancy.[2,3] Many people in India and all over the world suffer from the misconception that herbal products are totally safe and do not possess any side effects because they are derived from natural sources and have been used for many years.[4,5] Components of medicinal plants can alter the absorption and/or metabolism of conventional drugs leading to reduced efficacy or systemic drug toxicity.[6] Repaglinide is an oral insulin secretagogue of the meglitinide class. Due to lower risk of

hypoglycemia these agents are main option for some elderly patients. It is associated with 60% fewer hypoglycemic episodes compared with sulfonylureas.[7] The drug is completely (98%) metabolized in the liver and is excreted primarily through the bile. None of its major metabolites contribute the glucose lowering effect and the drug does not appear to accumulate with repeated dosing. Since, repaglinide has a short duration of action and is excreted independently of renal function; it may be suitable for use in patients with type 2 diabetes with renal impairment. Repaglinide is metabolized by CYP3A4 and CYP2C8 enzymes.[8] Curcumin is obtained from dried rhizomes of plant *Curcuma longa* (Zingiberaceae). It is widely used as a food additive in India from olden days onwards.[9] It is used in the treatment of number of ailments. Many drug interactions are a result of induction or inhibition of CYP enzymes. Herbal drugs that modulate intestinal and hepatic CYPs can alter the bioavailability and clearance of co-administered drugs. Several *in vitro* reports of curcumin proved that it is a CYP inhibitor, especially CYP3A4, CYP1A2 and CYP2C9.[10,11] The present study was designed to investigate the herb-drug interaction between repaglinide and curcumin because there is insufficient data available regarding the dietary supplements, nutrients and herbal interaction with repaglinide.

Materials and methods

Animals and diet:

Male albino rats of wistar strain weighing 200-280 g were purchased from Sainath Agencies, Hyderabad, India and used for the study after obtaining permission from the institutional animal ethical committee (CPCSEA Reg. No. IAEC/07/UCPSc/KU/2016). The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12 h light/dark cycle; at an ambient temperature of 25±5 ℃; 35-60 % relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum*.

Drugs and chemicals: Repaglinide and Ritonavir (internal standard) were the kind gift samples from Novartis, Hyderabad, India. Curcumin was obtained as a gift sample from Nisarg Biosciences, Hyderabad, India. Streptozotocin was supplied by Sigma-Aldrich, Bangalore, India. Glucose estimation kit was supplied by Adi diagnostics, Hyderabad, India. HPLC grade methanol was supplied by Merck, Mumbai, India. Water used for analytical purpose was double distilled, filtered by using direct-Quv Millipore and sonicated for removing air bubbles. All other chemicals used for study were of analytical grade.

HPLC analysis of repaglinide:

Serum repaglinide concentration was estimated by slightly modified reverse phase HPLC method of Seema *et al*.[12] The serum concentrations of repaglinide were quantified by a validated ultra fast liquid chromatography coupled with photodiode array detection (Shimadzu Corporation, Kyoto, Japan). The system consisted of binary LC-20AD pumps with a micro gradient mixer. RP C18 column, 250 mm×4.6 mm, 5 µm (Phenomenex Luna) was used at 35±2.0 ℃. All the operations and analysis of data obtained were controlled by lab solutions software. A mixture of methanol and distilled water in the ratio of 80:20 (v/v) used as mobile phase and delivered at a flow rate of 1 ml/min. The mobile phase was degassed by sonicator and filtered through 0.22 µm membrane filter. Then detection was carried out at a wavelength of 260 nm. The total run time for analysis was 10 min. 100 µl of test rat serum was taken in a polypropylene centrifuge tubes, then add 100 µl of internal standard (10 µg/ml) solution and vortex for 1 min. Then add 100 µl of methanol to precipitate the proteins. The mixture was vortexed for 1 min and centrifuged for 20 minutes at 3000 rpm. Clear supernatant was collected in another centrifuge tubes. 20 µl of aliquot was injected into the analytical column for analysis. Intraday and inter day precision were obtained from three levels of quality control samples of repaglinide. The precision and accuracy of the method was determined by taking quality control samples at three concentrations of 2, 10, and 100 µg/ml and all the samples were run in three

replicates. Intraday precision data was obtained in a single day by analyzing three sets of quality control samples, while the inter day data was obtained on three consecutive days by analyzing the quality control samples. The assay procedure was found to be precise and accurate. Limit of detection and limit of quantification of repaglinide was found to be 0.287374 µg and 0.957912 µg respectively. Hence the LOD and LOQ were found to be within the range of the analyzed levels in serum samples.

Experimental design

Pharmacodynamic and pharmacokinetic interaction study in normal rats:

After overnight fasting, rats were randomly divided into four groups (n=6). Group I administered orally with repaglinide, 0.2 mg/kg[13], Group II administered orally with curcumin, 80 mg/kg[11], Group III administered orally with curcumin (80 mg/kg PO) followed by repaglinide (0.2 mg/kg PO) for single dose interaction studies and Group IV administered orally with curcumin (80 mg/kg PO) for 7 days and on 8th day curcumin (80 mg/kg PO) followed by repaglinide (0.2 mg/kg PO) for multiple dose interaction studies. Through retro orbital plexus blood samples were collected by using heparinised capillary tubes, and immediately same volume of normal saline was replaced intra peritoneally. The blood was collected at time intervals of 0, 0.5, 1, 2, 4, 8, and 24 hrs in every group. Serum was separated after centrifugation at 8000 rpm for 15 min and the samples were stored at – 20 ℃ until analysis.[11]

Pharmacodynamic and pharmacokinetic interaction study in diabetic rats:

Induction of diabetes: Male albino wistar rats (200-280 g) were randomly selected and fasted overnight for study and diabetes was induced by IP injection of streptozotocin 55 mg/kg body weight, in freshly prepared citrate buffer (pH 4.5).[11] After 72 h, blood samples were collected from rats by retro orbital puncture and the serum was analyzed for glucose levels by peroxidase (POD) glucose oxidase (GOD) method (Trinder, 1969). The animals with blood glucose level >250 mg/dl were considered as diabetic and were used in the study.

Grouping of diabetic rats: After overnight fasting, rats were randomly divided into four groups (n=6). Group V administered orally with repaglinide, 0.2 mg/kg[13], Group VI administered orally with curcumin, 80 mg/kg[11], Group VII administered orally with curcumin (80 mg/kg PO) followed by repaglinide (0.2 mg/kg PO) for single dose interaction studies and Group VIII administered orally with curcumin (80 mg/kg PO) for 7 days and on 8th day curcumin (80 mg/kg PO) followed by repaglinide (0.2 mg/kg PO) for multiple dose interaction studies. Through retro orbital plexus blood samples were collected by using heparinised capillary tubes, and immediately same volume of normal saline was replaced intra peritoneally. The blood was collected at time intervals of 0, 0.5, 1, 2, 4, 8, and 24 hrs in every group. Serum was separated after centrifugation at 8000 rpm for 15 min and the samples were stored at – 20 ℃ until analysis.[11]

Calculation of pharmacodynamic and pharmacokinetic parameters:

Non compartmental pharmacokinetic analysis was carried out using phoenix winnonlin software. The parameters like C_{max}, T_{max}, AUC_{0ton}, AUC_{total}, $AUMC_{total}$, $t_{1/2}$, MRT, Cl and Vd were calculated. For the pharmacodynamic data, mean blood glucose levels and percentage reduction in blood glucose concentrations were determined. % glucose reduction at t hour = [(A-B) / A] × 100

A = mean glucose levels at t hour

B = mean glucose levels at 0 hour

Statistical analysis: All the results were expressed as mean ± SD. The data were statistically evaluated using one way ANOVA with Bonferroni post-test performed using Graph Pad Prism 7.01 software. Values corresponding to $p \leq 0.05$ were considered as significant.

RESULTS

Pharmacokinetic interaction study of curcumin and repaglinide combination in normal rats: The pharmacokinetic parameters in repaglinide treated group were calculated and showed C_{max} of 3.778±0.532

µg/ml, T_{max} of 1 hr and AUC_{0ton} of 23.06±3.184 µg.hr/ml in normal rats. Increase in C_{max}, AUC, and AUMC; and decrease in clearance and Vd was observed in single dose curcumin treated and multiple dose curcumin treated groups and was statistically significant when compared with repaglinide alone group. All the pharmacokinetic parameters were shown in table 1.

Pharmacokinetic interaction study of curcumin and repaglinide combination in STZ induced diabetic rats:

Pharmacokinetic parameters in repaglinide alone group were calculated and showed C_{max} of 7.398±0.655 µg/ml, T_{max} of 1.16±0.408 hr and AUC_{0ton} of 41.393±6.355 µg.hr/ml in diabetic rats. C_{max}, AUC, and AUMC were increased in single dose and multiple dose curcumin treated groups. Clearance and Vd was decreased and is statistically significant in single dose and multiple dose curcumin treated groups when compared with repaglinide alone group. All the pharmacokinetic parameters were shown in table 2.

Pharmacodynamic interaction study of curcumin and repaglinide combination in normal rats:

At each time point the mean blood glucose levels were calculated using glucose oxidase peroxidase method and the percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated in normal rats. The mean blood glucose levels and percentage glucose reduction was compared against repaglinide alone group in normal rats. Pretreated curcumin groups were found to have no significant effect on the diabetic control or the percentage reduction of glucose levels in normal rats. Mean blood glucose levels of three groups were shown in figure 1.

Pharmacodynamic interaction study of curcumin and repaglinide combination in diabetic rats:

At each time point the mean blood glucose levels were calculated using glucose oxidase peroxidase method and the percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated in diabetic rats. The mean glucose levels and percentage glucose reduction was compared against repaglinide alone group in diabetic rats. Pretreated curcumin groups were found to decrease the mean blood glucose levels and thus increase the percentage glucose reduction in both single dose and multiple dose exposure, with more statistical significance in multiple dose group $p < 0.05$. Mean blood glucose levels of all the diabetic groups were shown in figure 2.

DISCUSSION

DM is a chronic metabolic disorder and it also induces other diseases like neuropathy, retinopathy, cardiomyopathy, nephropathy, high oxidant stress and peripheral neuropathy. The main objective of this study is to determine the influence of curcumin on pharmacokinetics and pharmacodynamics of repaglinide in normal as well as in STZ induced diabetic rats. The factors which influence the CYP mediated metabolism either directly or indirectly are likely to be prominent for interaction. It may be due to induction or inhibition of CYP enzyme.[14] The inhibition of CYP enzymes can increase the plasma concentration of simultaneously administered drugs and enhance the pharmacological and toxicological effect. Whereas in case of induction of CYP enzymes, can lower the concentration of plasma of simultaneously administered drugs and reduce the therapeutic effect.[14] Herbal drugs which alter hepatic and intestinal CYPs can modify the bioavailability and clearance of administered drugs. Repaglinide is metabolized by CYP3A4 and CYP2C8 enzymes.[8] Curcumin was reported to inhibit several CYP enzymes like CYP3A4, CYP1A2 and CYP2C9.[10,11] In this study we determine the influence of curcumin on the pharmacokinetics and pharmacodynamics of repaglinide in normal and STZ induced diabetic rats. We found that pharmacokinetic parameters were changed in curcumin treated groups, but statistically more significant in multiple dose treated group. In normal rats, curcumin treated groups showed AUC_{total} 44.16±2.268 ($p<0.01$) and $AUMC_{total}$ 310.27±82.16 ($p<0.01$).

Table 1: Mean pharmacokinetic parameters of repaglinide in different groups of normal rats

PK Parameter	Repaglinide	Repaglinide+Cur (SDI)	Repaglinide+Cur (MDI)
C_{max} (µg/ml)	3.778±0.532	5.379±0.526**	6.124±0.692**
T_{max} (h)	1±0	1.16±0.408	1.33±0.516
AUC_{0-n} (h µg/ml)	23.06±3.184	37.23±6.165**	42.81±2.459**
AUC_{total} (h µg/ml)	23.18±3.143	38.09±6.783**	44.16±2.268**
$AUMC_{total}$	133.95±21.57	260.12±87.87*	310.27±82.16**
$t_{½}$ (h)	3.046±0.502	3.833±1.273	4.221±1.792
MRT (h)	5.772±0.402	6.683±1.314	7.012±1.759
CL (ml/h/kg)	9.13±1.729	4.45±0.52**	3.773±0.348**
Vd (ml/kg)	39.62±6.311	24.291±7.216**	23.559±11.898

All values are expressed as mean ± SD (n=6)
*p<0.05; **p<0.01 considered as significant when compared with repaglinide control

Table 2: Mean pharmacokinetic parameters of repaglinide in different groups of diabetic rats

PK Parameter	Repaglinide	Repaglinide+Cur (SDI)	Repaglinide+Cur (MDI)
C_{max} (µg/ml)	7.398±0.655	10.392±1.148**	15.513±1.473**
T_{max} (h)	1.16±0.408	1.16±0.408	1.33±0.516
AUC_{0-n} (h µg/ml)	41.393±6.355	61.866±11.938*	77.835±6.334**
AUC_{total} (h µg/ml)	42.393±6.542	63.669±12.808*	81.735±7.560**
$AUMC_{total}$	267.12±95.98	427.34±145.706	575.69±151.44*
$t_{½}$ (h)	3.908±1.691	4.324±1.141	5.325±1.437
MRT (h)	6.205±1.833	6.571±1.228	6.975±1.418*
CL (ml/h/kg)	4.575±1.694	2.819±0.501*	2.28±0.217*
Vd (ml/kg)	22.998±2.369	17.422±4.82*	16.394±3.416**

All values are expressed as mean ± SD (n=6)
*p<0.05; **p<0.01 considered as significant when compared with repaglinide control

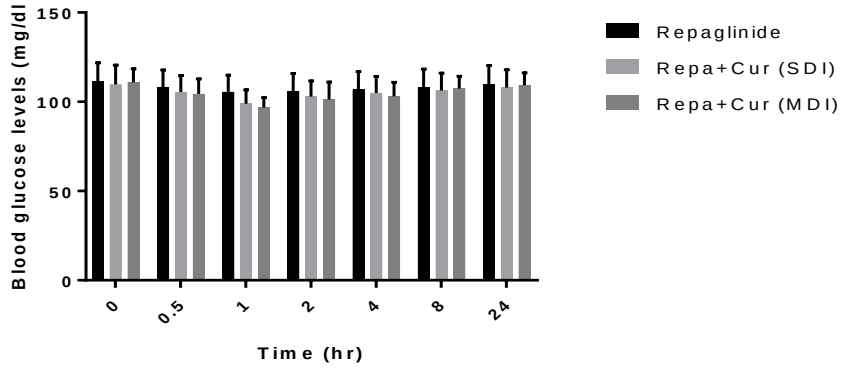

Figure 1: Effect of pretreatment of curcumin and repaglinide on blood glucose levels of normal rats

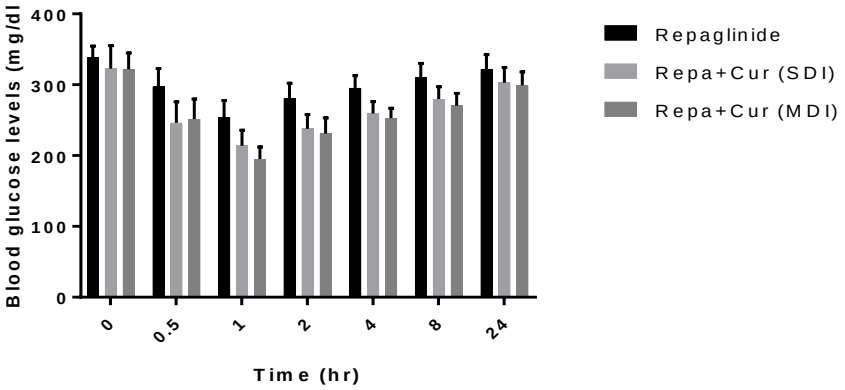

Figure 2: Effect of pretreatment of curcumin and repaglinide on blood glucose levels of STZ induced diabetic rats

In STZ induced diabetic rats, curcumin treated groups showed AUC_{total} 81.73±7.56 ($p<0.01$) and $AUMC_{total}$ 575.69±151.44 ($p<0.05$). There is increase in the T_{max} in both normal and STZ induced diabetic rats, may be due to change in the rate of absorption of repaglinide. The alteration in the metabolism of repaglinide may be due to, either by enhancing absorption or by inhibiting CYP3A4 enzyme which is responsible for metabolism of repaglinide.

We found there is no significant effect on the antihyperglycemic effect or the percentage reduction of glucose levels in curcumin treated normal rats. At 1 hour we observed maximum percentage glucose reduction in all the groups of normal rats. In STZ induced diabetic rats, we found decrease in mean blood glucose levels significantly and increase in the percentage of glucose reduction was observed at 1 hour in repaglinide, single dose and multiple dose curcumin treated groups (24.93±5.07%, 33.28±8.58% and 39.22±4.98% respectively). Percentage of glucose reduction was much significant ($p<0.01$) in multiple dose than in single dose treated groups ($p<0.05$). Increase in the AUC and AUMC in curcumin treated groups suggests that there is an inhibitory effect of curcumin on intestinal metabolism of repaglinide as curcumin has poor bioavailability.[15] Only in STZ induced diabetic rats the influence of curcumin was more effective by enhancing glycemic control, due to partly by increased pharmacokinetics of repaglinide and partly by antihyperglycemic activity of curcumin. There was more significant effect on the percentage glucose reduction in diabetic rats under multiple dose curcumin treatment and no significant effect in normal rats ($p>0.05$). Therefore, we observed the increased pharmacokinetic parameters of repaglinide more in multiple dose curcumin treated groups and the pharmacodynamic activity was improved significantly only in STZ induced diabetic rats in multiple dose curcumin treated group.

CONCLUSION:

The results showed that repaglinide level was increased due to metabolic inhibition of CYP3A4 in the presence of curcumin. It indicates that occurrence of drug interaction, which may be due to decreased metabolism of repaglinide. The variation is more prominent in multiple dose curcumin treated groups and the long term exposure to curcumin in diabetic condition should be controlled by repaglinide in rats. Although the combination has a beneficial effect in diabetic condition, it warrants further studies to check the applicability of this interaction in humans and determine the mechanisms involved.

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