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Research Article
Pharmacokinetic and Pharmacodynamic Interaction of Quercetin with Saxagliptin in Normal and Diabetic Rats

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Abstract

Background and Objective: People often take different herbs in combination with prescribed modern medication therapy in diabetes and such herbal preparations often contains quercetin that can inhibit cytochrome P450 (CYP)3A4. This enzyme is responsible for metabolizing saxagliptin, which is a potent and specific DPP-4 inhibitor used as anti-diabetic agent. The aim of the present study was that the quercetin may influence the both pharmacokinetic (PK) and pharmacodynamic (PD) interaction of saxagliptin, which could be particularly crucial, as any increment in its plasma levels may raise safety concerns. **Materials and Methods:** The effect of quercetin on the pharmacokinetics and pharmacodynamics of saxagliptin in normal as well as in streptozotocin (STZ) induced diabetic rats were studied. The data were statistically evaluated using one-way analysis of variance (ANOVA) followed by *post hoc* Dunnett's t multiple comparison test using GraphPad Prism 5. **Results:** In normal and diabetic rats, the combination of saxagliptin with quercetin, significantly increased all the pharmacokinetic parameters, such as Cmax, AUC0-n, AUCtotal, t1/2 and mean residence time and decreased the clearance, Vd, markedly as compared with the control group whereas, PD activity was also altered. **Conclusion:** The results suggesting that quercetin led to the PK/PD changes because of saxagliptin increased bioavailability and the inhibition of CYP3A4 enzyme. In conclusion, add on preparations containing quercetin may increase the bioavailability of saxagliptin and hence should be cautiously used.

Key words: Saxagliptin, quercetin, diabetic rats, herb drug interaction, pharmacokinetic, pharmacodynamic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of complementary therapies for treatment of diabetes is ever increasing and often remains unnoticed by a physician[1]. Diabetic patients often consume herbal preparations along with routinely prescribed antidiabetic agents[2]. As people often take different herbs in combination with prescribed modern medication so, there may be a chance of drug interaction. A drug interaction is defined either as increase or decrease of therapeutic effect of a specific drug caused by another substance, which may be another drug, plant or a dietary supplement, Thus leads to a potential for both pharmacokinetic (PK) and pharmacodynamic (PD) interaction[3,4].

Saxagliptin is a potent and specific inhibitor of DPP-4 (in comparison with other dipeptidyl peptidase enzymes). Saxagliptin largely metabolized by hepatic cytochrome P450 (CYP) 3A4 and 3A5 isoforms. With the exception of the primary hydroxylated metabolites of saxagliptin, which is 2 fold less potent than its parent molecule, metabolic products of hepatic biotransformation are minimally active and none appreciably contribute to either the therapeutic or the toxic effects of DPP-4 inhibitors[5].

Quercetin has been reported as anti-inflammatory, anti-oxidant, anti-carcinogenic and anti-diabetic. There are several *in vitro* reports of quercetin on inhibition of the CYP450s especially CYP3A4, CYP2C8, CYP2C9[6,7]. If you eat lots of vegetables and fruits, you'll get a fair amount of quercetin. Thus, there is a need to study the PK and PD interaction between saxagliptin and quercetin to avoid adverse effects. It may lead to change in the bioavailability of concomitant drug. Hence there is possibility of quercetin for the metabolic inhibition of saxagliptin. The aim of the present study was that the quercetin may influence the both pharmacokinetic (PK) and pharmacodynamic (PD) interaction of saxagliptin, which could be particularly crucial, as any increment in its plasma levels may raise safety concerns.

MATERIALS AND METHODS

Drugs and chemicals: Saxagliptin were obtained as a gift sample from Mylan laboratories, Hyderabad. Phytochemical quercetin was purchased from Yucca Enterprises, Mumbai. Streptozotocin (STZ) was purchased from HI Media Laboratories Pvt. Ltd, Mumbai. Acetonitrile (HPLC grade), potassium dihydrogen orthophosphate and orthophosphoric acid of analytical reagent grade (99.5%) were procured from Merck Specialties Pvt. Ltd, Mumbai, India. All other chemicals used were of analytical grade.

Maintenance of animals: Male albino rats of Wistar strain weighing 180-250 g were purchased from Mahaveera Enterprises, Hyderabad, India and used for the studies after obtaining the permission from the Institutional Animal Ethical Committee (Committee for the Purpose of Control and Supervision of Experiments on Animals Reg. No. IAEC/42/UCPSc/KU/2015). The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12:12 h light and dark cycle, at an ambient temperature of 25±5°C, 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum*.

Pharmacokinetic study

Grouping of normal rats and pretreatment: Rats were divided into two groups (n = 6). Group I was administered with saxagliptin (8 mg kg^{-1}, b.wt., p.o.) suspended in normal saline on the 8th day. Group II was pretreated with quercetin (20 mg kg^{-1}, b.w., p.o.) for 7 days and on the 8th day with saxagliptin (8 mg kg^{-1}) followed by quercetin. Before the collection of blood samples, animals were fasted for 16 h with water *ad libitum*. Blood samples were collected from retro-orbital vein puncture using heparinized capillary tubes at 0.5, 1, 2, 4, 6, 8 and 12 h. Serum was separated after centrifugation at 8000 rpm for 15 min and stored at -20°C until analysis[8-10].

Induction of diabetes in rats: Diabetes was induced by using STZ (55 mg kg^{-1}, b.wt., i.p.) in citrate buffer (pH 4.5) to the overnight fasted Wistar rats. After 72 h, blood samples were collected from rats by retro-orbital puncture and the serum was analyzed for glucose levels. Animals with blood glucose level >250 mg dL^{-1} were considered as diabetic and were used for the study[11].

Grouping of diabetic rats and treatment: Diabetic rats were divided into two groups (n = 6) and were treated, blood samples were collected as mentioned for normal rats. The HPLC analysis of saxagliptin in normal and diabetic pretreated rats. Serum saxagliptin concentration was determined by using reverse phase HPLC method[12]. The HPLC system was equilibrated with the mobile phase consisting of 0.05 M potassium dihydrogen orthophosphate (pH 4.5 adjusted with orthophosphoric acid), methanol, acetonitrile (60:20:20 v/v), at a flow rate of 0.6 mL min^{-1}, the photo diode array (PDA) detector was operated at a wavelength of 220 nm.

Pharmacodynamic studies

Effect of quercetin with saxagliptin on serum glucose in STZ induced diabetic rats: The STZ induced diabetic rats were fasted overnight and divided into 4 groups (n = 6). The animals of group I (diabetic control, normal saline), group II (saxagliptin, 8 mg kg^{-1}), group III (quercetin, 20 mg kg^{-1}) and group IV [quercetin (20 mg kg^{-1})+saxagliptin (8 mg kg^{-1})] were treated orally with the material mentioned in the parenthesis of the respective group. The effect of the quercetin, saxagliptin alone and their combinations on fasting blood glucose level was studied up to 12 h. Blood samples were drawn from the retro-orbital plexus of the rats at 0 (Initial fasting blood sample), 2, 4, 6, 8 and 12 h after the treatment. The samples were analyzed for blood glucose using glucose oxidase-peroxidase method[13,14].

Statistical analysis: The Pharmacokinetic parameters were calculated by using Kinetica TM software (version 4.4.1, Thermo Electron Corporation, USA). All values of pharmacokinetic and pharmacodynamic studies were expressed as Mean±SD. The data were statistically evaluated using one-way analysis of variance (ANOVA) followed by *post hoc* Dunnett's t multiple comparison test using GraphPad Prism 5 computer software. Values corresponding to p = 0.05 were considered as significant.

RESULTS

Pharmacokinetics of saxagliptin in normal and diabetic pretreated rats: Data in Table 1 are summarized the pharmacokinetic parameters of saxagliptin in different groups of normal and diabetic rats. In normal and diabetic pretreated rats, compared with the control group (given saxagliptin alone), the co-administration of quercetin significantly ($p<0.01$) increased Cmax (2.71 and 2.31 times), AUC0-n (4.86 and 4.33 times), AUCtotal (2.84 and 4.55 times), t1/2 (2.62 and 4.23 times), MRT (1.74 and 2.83 times), whereas, the clearance (3.146 and 2.906 times) and volume of distribution (1.57 and 1.99 times) of saxagliptin was decreased. The Tmax was not altered significantly in both normal and diabetic pretreated rats. Results in Fig. 1 represented the mean serum concentrations of control and treated groups of normal and diabetic rats. In the figure, X-axis represents time in hours after administration of saxagliptin and Y-axis represents mean serum concentrations of saxagliptin.

Pharmacodynamic study

Effect of quercetin and their combination on the hypoglycemic action of saxagliptin: The mean serum glucose level and percentage glucose reduction of antihyperglycemic study of pretreated diabetic rats is shown in Table 2. The data revealed that there is a maximum reduction of serum glucose level in combination of quercetin with saxagliptin pretreated groups (39.33%), when compared with standard (saxagliptin, 25.52%), quercetin (22.09%), alone pretreated groups at the 6th h, respectively. However, combination of quercetin with saxagliptin showed sharp decrease ($p<0.01$) in serum glucose levels at all the time points.

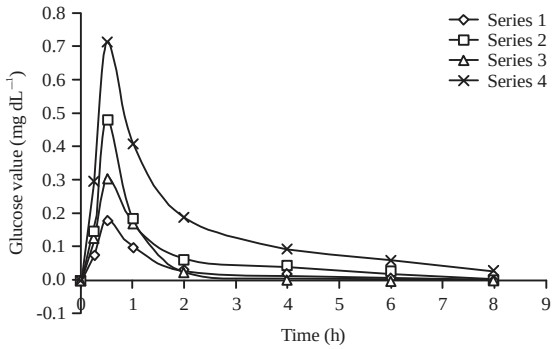

Fig. 1: Comparison of mean serum concentrations of saxagliptin in different groups of normal and STZ induced diabetic rats

Series 1: Normal rats treated with saxagliptin, Series 2: Normal rats treated with quercetin for 7 days and treated saxagliptin along with quercetin on 8th day, Series 3: Diabetic rats treated with saxagliptin, Series 4: Diabetic rats treated with quercetin for 7 days and treated saxagliptin along with quercetin on 8th day

Table 1: Mean pharmacokinetic parameters of saxagliptin in different groups of normal and STZ induced diabetic rats

	Saxagliptin		Saxagliptin+Quercetin	
Pk parameters	Normal	Diabetic	Normal	Diabetic
Cmax (μg mL^{-1})	0.176±0.08	0.307±0.12	0.478±0.003*	0.7106±0.04*
Tmax (h)	0.5	0.5	0.5	0.5
AUC0-n (μg mL^{-1} h^{-1})	0.105±0.048	0.280±0.04	0.511±0.083*	1.213±0.0062*
AUCtotal (μg mL^{-1} h^{-1})	0.196±0.02	0.282±0.052	0.557±0.044*	1.284±0.0083*
T½ (h)	0.763±0.3	0.491±0.242	2±0.04*	2.077±0.021*
MRT (h)	1.286±0.2	0.952±0.127	2.25±0.016*	2.7±0.033*
Vd (mL)	90.4±7.5	44.8±4.5	57.4±2.5*	22.5±1.7*
CL (mL min^{-1})	38.7±1.4	18.6±1.1	12.3±0.1*	6.4±0.3*

Values are expressed as Mean±SD (n = 6), *$p<0.01$ considered as significant when compared with control groups

Table 2: Comparison of mean serum glucose levels and percentage reduction of serum glucose level of group II, III and IV with group I in STZ induced diabetic rats

Group No.	Treatment	Dose (mg kg^{-1})	Blood glucose level (mg dL^{-1}) at different hours					
			0	2	4	6	8	12
I	Control	-	334.5±1.6	332.8±1.6 (0.49%)	332.3±1.4 (0.66%)	332.7±1.1 (0.54%)	333.2±1.2 (0.39%)	334.3±1.8 (0.07%)
II	Saxagliptin	8	342.5± 2.4	281.6±3.9 (17.79%)**	226.3±4.6 (33.93%)**	255.1±11.3 (25.52%)**	291.5±4.9 (14.88%)**	331.1±1.3 (3.35%)*
III	Quercetin	20	326.4±2.8	300.6±5.1 (7.9%)**	252.3±4.7 (19.64%)**	254.3±1.7 (22.09%)**	285.4±3.6 (12.56%)**	313.2±7.4 (4.04%)*
IV	Quercetin+Saxagliptin	20+8	355.2±5.1	293.6±4.5 (17.34%)**	242.3±5.2 (31.78%)**	215.5±2.5 (39.33%)**	238.2±7.2 (32.94%)**	281.2±6.4 (20.83%)**

Values are expressed as Mean±SD (n = 6), *p<0.05, **p<0.01 considered as significant when compared with group I at respective time interval

DISCUSSION

In normal and STZ induced diabetic rats, combination of saxagliptin with quercetin leads to a significant increase in PK parameters such as Cmax, AUC0-n, AUCtotal, t1/2 and MRT. This may be due to alteration in the metabolism of saxagliptin either by enhancing absorption or by inhibiting CYP3A4/A5 responsible for saxagliptin metabolism. Quercetin is one such naturally occurring dietary flavonoid, ubiquitously present in many antidiabetic herbal preparations and even in black tea, red wine and various fruit juices[15]. Quercetin is also reported to inhibit the activity of CYP3A4 *in vitro*[16,17].

This indicates that the decreased volume of distribution may not be due to displacement of saxagliptin by quercetin. As there is no plasma protein binding interactions between quercetin and saxagliptin, the decreased volume of distribution may be due to metabolic inhibition of saxagliptin by quercetin. The present investigations are in accordance with the earlier *in vitro* studies of quercetin metabolic inhibition on CYP3A4 enzyme in rat and human[16,17].

The mean serum concentration of saxagliptin in diabetic rats was found more than normal rats, due to elevated CYP enzyme expression in diseased condition, since increased reactive oxygen species production and oxidative stress occur in diabetes which effect the metabolism[5]. There is no change in Tmax of saxagliptin in both normal and diabetic rats indicating that there is no alteration in rate of absorption of saxagliptin and the serum affinity of saxagliptin for albumin is 99.5% bound.

The enhance in hypoglycemic action by simultaneous administration of saxagliptin with quercetin was more in diabetic rats than when the drugs were used singly and with the control group, which suggests the enhancement of glucose reduction capacity of saxagliptin in diabetic rats along with quercetin. Influence of quercetin were effective in improving PDs (glycemic control) in diabetic rats, which indicates that the alteration might be partly because of improved PKs of saxagliptin and partly because of antihyperglycemic activity of quercetin. Thus, the significant improvement in PK parameters and in PDs of saxagliptin was more observed in the combination of saxagliptin with quercetin.

CONCLUSION

The present study indicated that quercetin affects the metabolism of saxagliptin, possibly by the inhibition of CYP2C9 and CYP3A4. Combination of saxagliptin with

quercetin considerably enhances the glucose lowering effect of quercetin and saxagliptin. Hence, saxagliptin doses may require special attention if used along with quercetin containing herbal preparations to avoid complications.

SIGNIFICANCE STATEMENTS

This study discovered the effect of quercetin on the pharmacokinetics and pharmacodynamics of saxagliptin in normal as well as in streptozotocin induced diabetic rats. That can be beneficial for patients with diabetes, frequently use herbal or nutrient supplements and/or concomitant medications with their treatment. This study helps the researchers to uncover the critical areas of dose monitoring in diabetic patients that many researchers were not able to explore. Thus a new theory on herb-drug interaction may be arrived at.

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