EFFECT OF DIABETES MELLITUS ON THE PHARMACOKINETIC BEHAVIOR OF GATIFLOXACIN IN SPRAGUE-DAWLEY RATS

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ABSTRACT: Effects of diabetes mellitus induced by streptozotocin (DMIS) on the pharmacokinetics of Gatifloxacin were investigated after i.v and oral administration (50mg/mg) to control Sprague-Dawley rats and DMIS rats (at 7th and 29th days after administration of streptozotocin (55mg/kg). After i.v administration to DMIS rats, there was no significant difference in clearance, $t_{1/2}$, V_d and MRT last. But after oral administration of gatifloxacin to DMIS rats Cmax was significantly increased. This could be supported with the marginal increase in AUC and an increase in bioavailability of drug in chronic (78.7% increase) as well as acute conditions (75.3% increases). Streptozotocin induced toxicity did not influence considerably on the pharmacokinetics of gatifloxacin.
Key words: Diabetes mellitus, Pharmacokinetics, Gatifloxacin

INTRODUCTION

Gatifloxacin is a synthetic 8-methoxy-7- (3-methylpiperazinyl) fluoroquinolone antibacterial agent for oral or intravenous administration. Gatifloxacin rapidly inhibits DNA synthesis by promoting cleavage of bacterial DNA in the DNA enzymal complexes of DNA gyrase and type-IV topoisomerase, resulting in rapid bacterial death[1,2,3] . Gatifloxacin demonstrates increased activity against *Staphylococcus aureus*; *Staphylococcus epidermidis*, *Streptococcus pyogenes*, group B streptococcus, antibiotic resistant *Streptococcus pneumoniae* and viridans group streptococcus[4,5,] It also has excellent activity against a number of gram-negative enteric and respiratory pathogens including *Haemophilus influenzae*, *Enterobacter* spp., *Klebsiella* spp., *Serratia marcescens*, *Legionella pneumophila*, and *Proteus* spp. [6,7]. As a result, the Food and Drug Administration approved it for the treatment of community-acquired pneumonia, acute bacterial exacerbations of chronic bronchitis, sinusitis, and urinary tract infections in adults [8]. The efficacy of gatifloxacin in the treatment of nosocomial pneumonia is currently being assessed in clinical trials [9].

The diabetic state has been found to be associated with various perturbations of gastrointestinal tract which include gastroparesis (delayed gastric emptying) [10] gastric dysrrythmias [11], decreased gastric secretory functions [12,13] disordered gastric motor functions [14], dyspepsia [10], gastrointestinal dysmotility [15] and delayed intestinal transit[16] .

Diabetic nephropathy is a leading cause of end stage renal disease (ESRD) including complications like hemodynamic alterations in microcirculation (glomerular hyperfiltration, increases glomerular capillary pressure) [17] and structural changes in the glomerulus (increased extra cellular matrix, basement membrane thickening, mesangial expansion, fibrosis). Diabetes is also associated with proteinuria and micro albuminuria [18,19,20,]. All these factors contribute to a progressive decline in glomerular filtration rate.

Many diabetic patients develops serious complications during the course of the disease, including cardiovascular disorders, nephropathy, neuropathy and retinopathy [21]. Animal models of insulin dependent diabetes mellitus induced by administration of several chemicals, principally alloxan, streptozotocin and zinc chelators, were reported [22,23]. In rats with diabetes mellitus induced by streptozotocin (DMIS) bile flow was decreased and bile composition was altered [24], and hepatotoxicity [25] and impaired kidney function [26,27] were observed. Glucoronidation and sulfation were also profoundly affected by DMIS in rats [28]. Hence the pharmacokinetics and pharmacodynamics of drugs could be altered in DMIS rats.

It was reported [25] that in DMIS rats, a return to preinjection bile acid levels and bile flow rates occurred by 15th day after streptozotocin administration. These results suggest that streptozocin-induced hepatotoxicity may contributes to the results of biliary excretion studies performed less than 2 weeks after streptozotocin administration .It was also reported [25] that in DMIS rats, the complication of any toxic effects of streptozotocin was minimized by carrying out experiments 4-5 weeks after the initial streptozotocin injection. Hence the present studies were performed at 7th and 29th days after streptozotocin administration. In diabetes mellitus patients bacterial infections are more susceptible [29], hence gatifloxacin was chosen in the present study.

The aim of this paper is to report pharmacokinetic changes of gatifloxacin after both i.v and oral administration to DMIS rats at 7th and 29th days after single tail vein administration of streptozotocin.

MATERIALS AND METHODS

Chemicals

Gatifloxacin (Batch no. 20020604) was purchased from Jiangyin Yongda Chemicals Ltd, China. Citric acid (Batch no. NL 3998 5602) and D-Glucose (Batch no. EL 663 5402) were purchased from Qualigens Fine Chemicals, Bombay, India.Tri-sodium citrate dihydrate purified (Batch no. ML 3M532307) was purchased from Merck Ltd, Bombay, India. Streptozotocin, minimum 98% HPLC S0130–(Batch no. 015K1279) was purchased from Sigma-Aldrich Chemical Company (St.Louis, MO, USA). Other chemicals were of reagent grade or HPLC grade, and therefore were used without further purification.

Animals

Male Sprague-Dawley rats (weight 180-260 gms) were obtained from the Animal House Facility, Wockhardt Research Centre, and Aurangabad. The rats were divided into two groups, control rats and DMIS rats.

Body weight gain decreased significantly in DMIS rats at 29th day. Therefore the rats with small body weight were chosen as control for 29th day. All rats were provided with rodent pellet feed (Amrut Laboratory Animal Feed-Supplied by Pranav Agro Industries, Pune, B.No: 560831) and water ad libitum and maintained in a light controlled room (light-06.00—18.00, dark 18.00—06.00), kept at a temperature of 22±2°C and a relative humidity of 55±5% (Animal House Facility, Wockhardt Research Centre, Aurangabad). Rats were housed in individual polypropylene cages with SS top grill containing autoclaved rice husk .The protocol of this study was approved by an Institutional Animal Ethics Committee (IAEC), Wockhardt's Animal Ethics Committee under the guidelines of CPCSEA, Ministry of Environment, The Govt of India.

Induction of diabetes mellitus in rats by streptozotocin injection

Diabetes was induced experimentally in overnight fasted rats by a single tail-vein injection of freshly prepared streptozotocin (dissolved in citrate buffer of pH 4.5) at a dose of 55mg/kg. The rats were given 5% dextrose solution ad libitum for 12 hrs to prevent the onset of transient hypoglycemia. The same volume of citrate buffer (pH 4.5) was injected to control rats.

Intravenous study

The study rats were kept for overnight fasting in individual cages provided with SS bottom grills and water ad libitum. Before the starting of experiment the fasted blood glucose level was measured with GLU Flex^{R} reagent cartridge (adaptation of hexokinase-glucose-6-phosphate dehydrogenase method) by autoanalyser (DADE BEHRING approved by USFDA) and the rats showing blood glucose level more than 250 mg/dl were included for the study.

At 7th (acute study) and 29th (chronic study) days after administration of streptozotocin, Gatifloxacin of pH 9.0-9.5 (dissolved in MilliQ water with the help of 0.1N NaOH) at a dose of 50mg/kg was injected (0.5ml/200 g body weight) via the tail-vein (n=6 for 7th and 29th days each). Approximately 0.3ml aliquot of blood sample was collected from each animal from the retro-orbital plexus at 0.25hrs, 0.5hrs, 1hr, 2hrs, 4hrs, 6hrs and 8hrs.immediately after collection the blood samples were kept at bacteriological incubator (Remi Instruments-RI 12S) at 37°C for 10minutes.Blood samples were centrifuged immediately at 8000rpm at a temperature of 18-22°C for 10 minutes by Hermle-Z323K Centrifuge machine. The separated serum was pooled from 3 animals for each time point and stored at in a -60°C freezer (Thermo Electron Corporation-VXE490) until LC-MS analysis of Gatifloxacin.

Rats for control Pk study (n=6) were selected according to the weight range of DMIS rats chosen for both acute and chronic Pk studies. Other procedures were similar as those in the above-mentioned I.V study.

Oral study

Gatifloxacin of pH 9.0-9.5 (dissolved in MilliQ water with the help of 0.1N NaOH) at a dose of 50mg/kg was injected (0.5ml/200g body weight) to the selected DMIS rats (n=6 for both 7th and 29th days) and to control rats (n=9). Other procedures were similar as those in the I.V study.

LC-MS/MS analysis

The concentration of gatifloxacin in the plasma samples was analyzed by the LC-MS/MS system (Sciex MS–API 3000) method developed by wockhardt research laboratory. Plasma calibration standards were prepared with the serial dilution of standard solution of 1mg/ml gatifloxacin with rat plasma to obtain 40,20,10,5,2.5,1.25,0.625,0.312 µg/ml strengths.

A100µl aliquot of biological sample was deproteinized with a 400µl aliquot of acetonitrile. After vortex mixing for 30 seconds the tubes were then centrifuged at 8000rpm at 4-6°C for 10 minutes. Resulted supernatant was given for LC-MS/MS analysis. The linearity of plasma calibration curve was found to be 0.9999.
The mobile phase, 0.05 aqueous Tri-Fluoro acetic acid (Merck-Schuchardt-B.No-S3819160 323) and acetonitrile (Rankem-B.No-R073L05) were run at flow rate of 0.25ml/min. A reversed-phase (C18) YMC-ODS-AM-150x2.0mm ID and 5µm size with guard column was used. The retention time was approximately 2.0 minutes. Detection was done by 3 quadrapole MS detector. The data was analyzed by Analyst 1.4.1 software.

Pharmacokinetic analysis

WinNonlin software was used to calculate the following pharmacokinetic parameters based on model independent method: Cmax, Tmax, area under the plasma concentration-time curve from time zero to time last measurable concentration (AUC_{0-t}), terminal half-life ($t_{½}$), area under the plasma concentration-time curve from time zero to time infinity ($AUC_{0-\alpha}$), volume of distribution (Vd), clearance (Cl), mean residence time (MRT).

Statistical analysis

A *P*-value less than 0.05 was considered to be statistically significant using the t-test between the two means for the unpaired data. All the data are expressed as mean ± standard error mean (SEM).

RESULTS

Pharmacokinetics of gatifloxacin after i.v administration

The plasma concentration–time profile of gatifloxacin after i.v administration to control rats and DMIS rats on 7th and 29th days is shown in **Figure 1** and some relevant pharmacokinetic parameters are listed in **Table 1.** After i.v administration of gatifloxacin to DMIS rats there was no significant difference in clearance, $t_{1/2}$, V_d and MRT last.

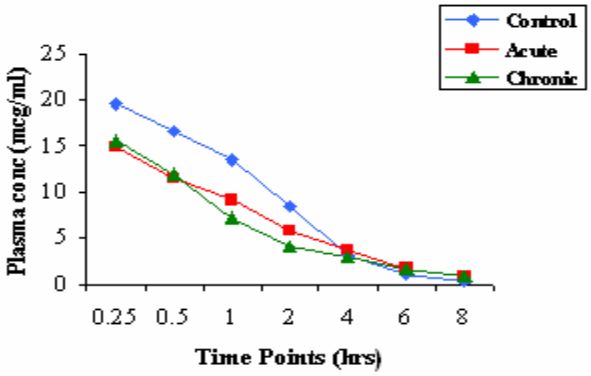

Figure: 1. Plasma concentration-time profile of gatifloxacin after 1 min i.v infusion at a dose of 50mg/kg to control rats (n=6) and DMIS rats (n=6) at the acute (7th day) and chronic (29th day) conditions.

Table: 1 Mean (± SEM) pharmacokinetic parameters of gatifloxacin after i.v administration of a dose of 50mg/kg to control and DMIS rats at 7th and 29th days (n=6).

Parameters	7th day		29th day	
	Control	DMIS	Control	DMIS
Initial body weight (g)	190.4 ± 3.95	197.67 ± 5.87	190.4 ± 3.95	197.67 ± 5.87
Final body weights (g)	267.92 ± 6.87	201.63 ± 17.44	371.44 ± 28.35	184.24 ± 30.83
Blood glucose (mg/dl)	78.30 ± 3.51	513.2 ± 54.17 *	78.30 ± 3.51	497.1 ± 28.65 *
Cmax (mcg/ml)	23.00 ± 2.480	20.72 ±11.03	23.00 ± 2.480	20.39 ± 2.520
Tmax (hr)	0.00 ± 0.00	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
AUC_{0-t} (mcg.hr/ml)	44.03 ± 2.610	37.07 ± 3.025	44.03 ± 2.610	31.76 ± 5.170
$t_{1/2}$ (hr)	1.30 ± 0.07	1.81 ± 0.14	1.30 ± 0.07	2.49 ± 0.87
$AUC_{0-\alpha}$ (mcg.hr/ml)	44.69 ± 2.720	39.28 ± 3.290	44.69 ± 2.720	35.07 ± 4.65
Vd (L/kg)	2.10 ± 0.01	3.36 ± 0.47	2.10 ± 0.01	5.34 ± 2.29
Clearance (L/hr/kg)	1.12 ± 0.06	1.27± 0.07	1.27± 0.06	1.43 ± 0.13
MRT last (hr)	1.86 ± 0.01	2.34 ± 0.35	1.86 ± 0.01	2.29 ± 0.21

* DMIS group was significantly different (P<0.05) from respective control group.
** DMIS group was significantly different (P<0.01) from respective control group.

Pharmacokinetics of gatifloxacin after oral administration

The plasma concentration–time profile of gatifloxacin after oral administration to control rats and DMIS rats on 7th and 29th days is shown in **Figure 2** and some relevant pharmacokinetic parameters are listed in **Table 2.** After oral administration of gatifloxacin to DMIS rats Cmax was significantly increased on 7th day (131% increase) and on 29th day (105% increase) when compared to control. This resulted in marginal increase in AUC and an increase in bioavailability on 7th day and on 29th day.

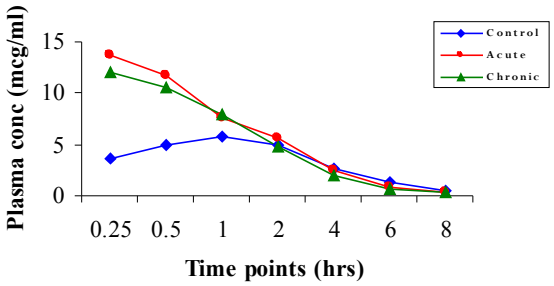

Figure.2. Plasma concentration-time profile of gatifloxacin after oral administration at a dose of 50mg/kg to control rats (n=6) and DMIS rats (n=6) at the acute (7th day) and chronic (29th day) conditions.

Table: 2 Mean (± SEM) pharmacokinetic parameters of gatifloxacin after oral administration of a dose of 50mg/kg to control and DMIS rats at 7th and 29th days (n=6).

Parameter	7th day		29th day	
	Control	DMIS	Control	DMIS
Initial body weight (g)	236.2 ± 19.55	231.76 ± 12.88	190.4 ± 3.95	197.67 ± 5.87
Final body weights (g)	273.68 ± 20.89	203.87 ± 14.52	371.44 ± 28.35	184.24 ± 30.83
Blood glucose (mg/dl)	94.33 ± 3.678	513.2 ± 54.17 *	78.30 ± 3.51	497.1 ± 28.65 *
Cmax (mcg/ml)	5.89 ± 0.92	13.66 ± 1.16**	5.89 ± 0.92	12.1 ± 0.28**
AUC_{0-t} (mcg.hr/ml)	22.29 ± 2.04	27.95 ± 2.31	22.29 ± 2.04	25.02 ± 1.05
$t_{1/2}$ (hr)	1.82 ± 0.15	1.46 ± 0.13	1.82 ± 0.15	1.44 ± 0.03
$AUC_{0-\alpha}$ (mcg.hr/ml)	23.77± 1.844	28.74 ± 2.585	23.77 ± 1.844	25.63 ± 1.135
Vd (L/kg)	5.70 ± 0.96	3.67 ± 0.01	5.70 ± 0.96	4.07± 0.26
Clearance (L/hr/kg)	2.13 ± 0.17	1.75 ± 0.15	2.13 ± 0.17	1.95 ± 0.08
MRT last (hr)	2.77 ± 0.03	2.09 ± 0.08**	2.77 ± 0.03	2.04 ± 0.09**

* DMIS group was significantly different ($P<0.05$) from respective control group.
** DMIS group was significantly different ($P<0.01$) from respective control group.

DISCUSSION

Induction of experimental diabetes mellitus was evident in DMIS rats based on significantly greater higher blood glucose values (Table 1-2) significantly greater 24 hr urine out put and significantly smaller body weight gain (Table 1-2) The significantly greater urine output in DMIS rats was also reported from other rat studies [30,26,31,25]

The plasma concentration–time profiles of gatifloxacin (both i.v and oral) were compared in control, acute diabetic and chronic diabetic rats as depicted in Figure 1 and Figure.2. After I.V administration of gatifloxacin to DMIS rats there was no significant difference in clearance, $t_{1/2}$, V_d and MRT last. But after oral administration of gatifloxacin to DMIS rats, Cmax was significantly increased. This could be supported with a marginal increase in AUC and an increase in bioavailability in chronic and acute conditions of diabetes. Hence, it can be hypothesized that the major mechanism behind the increase in the Cmax and AUC of gatifloxacin was due to an increase in the intestinal absorption. An increase in intestinal absorption of orally administered gatifloxacin may be attributed to delayed intestinal transit time reported in streptozotocin-induced rats [16] , which led the drug to remain in contact for prolonged time in jejunal portion of intestine.

Evidences suggest that the P-glycoprotein may regulate the absorption of fluoroquinolones. [32,33]. Ooie T et al reported that, plasma concentrations of grepafloxacin and levofloxacin after intravenous and intra intestinal administration were increased by cyclosporin A, a P-glycoprotein inhibitor, in rats. In addition, the accumulation of quinolones into the brain is low, which may be caused either by the relative low influx permeability at the blood-brain barrier and blood-cerebrospinal fluid barrier and/or by active P-gp efflux at both barriers [34]. Therefore, it can be hypothesized that P-glycoprotein may be a determinant factor in the absorption of gatiflloxacin also.

P-gp acts as a transmembrane pump, which removes drugs from the cell membrane and cytoplasm. ATP hydrolysis provides the energy for active drug transport, which can occur against steep concentration gradients. Damage to an efflux transporter may lead increase in absorption of drugs that are P-gp substrates. Hence it can be postulated that the possibility of P-gp damage due to the long standing oxidative stress induced by both streptozotocin and hyperglycemia resulted in a significant increase in Cmax in acute Diabetic (131% increase) and in chronic diabetic (105% increase).

CONCLUSION

After oral administration of gatifloxacin to DMIS rats Cmax was significantly increased on 7th day (131% increase) and on 29th day (105% increase) when compared to control. This resulted in marginal increase in AUC and an increase in bioavailability on 7th day and on 29th day. This could be supported with a marginal increase in AUC and an increase in bioavailability in chronic and acute conditions of diabetes. The major mechanism behind the increase in the Cmax and AUC of gatifloxacin may due to an increase in the intestinal absorption and delayed transit time reported in streptozotocin-induced rats [16].

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