

Serum Level Profile and Pharmacokinetic Parameters of Single Oral Dose of Metronidazole in Type II Diabetic Patients

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Abstract

Many pathophysiological processes can affect the pharmacokinetic properties of drugs in people with diabetes. The present study was designed to evaluate the influence of diabetes mellitus (DM) on the pharmacokinetic parameters of metronidazole administered as single oral dose. Twelve healthy volunteers and twelve diabetic patients were enrolled in the present study. On day 1, a single oral dose of metronidazole 500 mg was administered orally to all participants at 9:00 am after a 10-hour fasting. Over the following 48 hours, blood samples were taken at frequent intervals and serum metronidazole concentrations were measured by a high-performance liquid chromatography method for assessment of pharmacokinetics of metronidazole. The data indicated that maximum serum concentration (C_{max}) and K_{elim} were significantly decreased ($P < 0.05$) in diabetic patients compared with that reported in healthy subjects (25.73% and 31.51% respectively). Meanwhile, the values of time to reach maximum peak (T_{max}), AUC_{last} , AUC_{total} , and half life ($T_{1/2}$) were significantly increased ($P < 0.05$) compared with those reported in healthy subjects (20.69%, 33.65%, 30.13%, and 20.689% respectively). In conclusion, diabetes mellitus affects some of the pharmacokinetics values of orally administered metronidazole.

Key words: Diabetes mellitus, Metronidazole, Pharmacokinetic, Serum Level

الخلاصة

من المعروف ان لكثير من الحالات المرضية تأثير واضح على حركية بعض الأدوية في جسم المريض، وبناء على ذلك تم تصميم هذه الدراسة لتقييم تأثير النوع الثاني من داء السكري على معايير حركية الدواء لعقار الميترونيدازول ٥٠٠ ملغم عن طريق الفم. أجريت الدراسة على ١٢ مريض بالنوع الثاني من داء السكري و ١٢ شخصاً من الأصحاء. في اليوم الأول تم إعطاء ٥٠٠ ملغم ميترونيدازول كجرعة منفردة عن طريق الفم الى جميع المشاركين في الساعة التاسعة صباحاً بعد عشر ساعات من الصيام. وخلال الساعات الثمانية والأربعين اللاحقة تم سحب عينات من الدم خلال فترات محددة وقياس مستوى الميترونيدازول في مصل الدم بواسطة تقنية HPLC، وتم احتساب معايير حركية الدواء خلال هذه الفترة. أظهرت النتائج بأن المرض قد تسبب في خفض قيمة C_{max} و K_{elim} مقارنة بالأصحاء بينما أدت الى زيادة قيم T_{max} ، AUC_{last} ، AUC_{total} و $T_{1/2}$. وعليه يمكن الاستنتاج بأن لداء السكري بعض التأثيرات الجدية على معايير حركية الدواء لعقار ميترونيدازول عندما يعطى كجرعة منفردة عن طريق الفم.

Introduction

Many pathophysiological processes can affect the pharmacokinetic properties of drugs in people with diabetes⁽¹⁾. Patients with diabetes have higher rates of cardiovascular, renal, gastrointestinal, neurological, and thyroid diseases and ophthalmological complications compared with individuals without diabetes. All may increase the chance of having drug-disease interactions⁽²⁾. Some physiological disorders such as gastroparesis, decreased plasma albumin level, elevated plasma free fatty acid level, glycosylation of plasma proteins and changes in the hepatic microsomal cytochrome P-450 (CYP) contents were reported to occur in diabetes mellitus patients⁽³⁾; these changes could alter

the pharmacokinetics and hence the pharmacodynamics of drugs in such patients⁽⁴⁾. The extent of absorption of metoclopramide administered orally to diabetic patient with gastroparesis fell within the range of values reported in healthy subjects^(5,6). Glipizide was found to be completely absorbed when administered to patients with type II diabetes mellitus, just like in non-diabetic subjects^(4,7). In contrast, absorption of tolazamide was 26% slower in diabetic patients with asymptomatic autonomic neuropathy than in healthy subjects⁽⁸⁾. Metronidazole is used widely in diabetic patients for treatment of diabetic foot infections (DFIs).

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There is a wealth of information regarding the pharmacokinetic parameters of metronidazole in healthy subjects; however, limited information is available about this issue in diabetic patients. Since the metronidazole is often used to treat polymicrobial infections in diabetics⁽⁹⁾, the influence of the disease processes and consequent complications on the pharmacokinetic parameters of drugs, including metronidazole, should be systematically evaluated. The present study was designed to evaluate the influence of diabetes mellitus on the pharmacokinetics of metronidazole administered as single oral dose.

Subjects and Methods

Twelve healthy volunteers and twelve diabetic patients were enrolled in the present study; all have an age of 55 ± 10 years and with body weight range for each of 50 ± 5.0 kg and 75.0 ± 10.0 kg respectively. All healthy volunteers show a normal medical history and revealed no pathological abnormalities on clinical and biochemical examination. Meanwhile, all patients were selected for having type II diabetes mellitus for at least 5 years and have been treated with single daily dose of Glibenclamide 5mg daily and Metformin 500 mg three times daily. All patients had serum transaminase concentrations less than twice the upper limit of the laboratory reference range and a normal serum creatinine (<120 mmol /L). Written informed consent was obtained from each subject and the clinical protocol was approved by the Human Ethics Committee of the Iraqi Ministry of Health. All subjects were nonsmokers and were instructed not to drink caffeine or alcohol containing beverages for at least 10 hours before and during the study day. The study was performed according to an open, randomized clinical study design. Following a 5-day screening period, healthy volunteers and patients with type II diabetes were enrolled in the study. On day 1, a single oral dose of metronidazole 500 mg (Flagyl[®] Aventes) was administered orally to all participants at 9:00 am after a 10-hour fasting. Blood samples were taken at zero-time and at frequent intervals over a period of 48 hours following the administration of metronidazole (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, and 48.0 hours) and serum metronidazole concentrations were measured by a high-performance liquid chromatography method for assessing metronidazole pharmacokinetics. Stock solution of a Metronidazole (reference standard) (1mg/ml) was prepared by dissolving 100 mg in 100 ml of methanol. Working standard solutions were

prepared from the stock solution by sequential dilution with methanol to yield final concentrations of 1.5, 15, 75, 150, 225, and 300 $\mu\text{g/ml}$. Samples for the preparation of standard curve were prepared by mixing blank serum (specially prepared for this purpose) with different concentrations of standard Metronidazole solution to get the final required serum Metronidazole concentrations (0.15, 1.5, 7.5, 15.0, 22.5, and 30.0 $\mu\text{g/ml}$). After precipitation of protein by addition of 10% zinc sulfate, samples were vortexed, placed in refrigerator for 15 min and centrifuged at 3000 rpm for 10 min and 200 μl of supernatant was injected into the HPLC column for determination of serum levels⁽¹⁰⁾. The analyses were performed using HPLC system composed of a smart line pump 1000 and smart line U.V. detector 2500 connected to smart line manager 5000; the separation was performed on HPLC column (Eurospher-100 C18; 5 μm , 250 x4.5 mm). Drug analysis data were acquired and processed using CLASS-VP (v.6.2) software running under Windows 98 on a Pentium PC. The mobile phase consists of methanol: water in a ratio of 25:75 at a flow rate of 1 ml/min. The wave length was set at 276 nm⁽¹¹⁾. The results were expressed as mean $\pm$ SD. The data were statistically evaluated utilizing un-paired Student's *t*-test. Values with $P < 0.05$ were considered significantly different. Calculation of the pharmacokinetic parameters is performed utilizing the computer software kinetica PK-PD analysis version 5.0 (Microsoft-programs).

Results

The raw data for each group was presented in tables 1 and 2. The effects of diabetes mellitus (as a disease) on the absorption of Metronidazole were shown in figure 1 and table 3. The data in table 3 indicated that the value of maximum serum concentration (C_{max}) and K_{elim} were significantly decreased ($P < 0.05$) in diabetic patients compared with that reported in healthy subjects (25.73% and 31.51% respectively). Meanwhile, the values of time to reach maximum peak (T_{max}), AUC_{last} , $\text{AUC}_{\text{total}}$, and half life ($T_{1/2}$) were significantly increased ($P < 0.05$) compared with those reported in healthy subjects (20.69%, 33.65%, 30.13%, and 20.689% respectively).

Table 1: The pharmacokinetic parameters of orally administered 500 mg Metronidazole in healthy subjects.

Healthy Volunteers	C _{max} µg/ml	T _{max} hr	AUC _{last} µg hr ml ⁻¹	AUC _{total} µg hr ml ⁻¹	K _{elim} hr ⁻¹	T _{1/2} hr
V1	7.2	1.5	52.36	52.94	0.092	7.52
V2	7.16	1.5	46.90	49.37	0.058	11.99
V3	7.8	1.5	44.57	45.25	0.086	8.09
V4	7.86	1.5	4.66	47.39	0.083	8.39
V5	7.1	1.0	49.49	50.64	0.076	9.15
V6	7.26	1.5	43.55	44.46	0.075	9.23
V7	8.2	1.5	40.71	41.25	0.087	7.94
V8	7.56	1.5	45.0	49.58	0.047	14.86
V9	7.65	1.5	42.07	43.06	0.073	9.45
V10	7.96	1.5	72.59	79.04	0.05	13.67
V11	6.58	0.5	43.0	49.60	0.072	10.56
V12	8.58	2.5	45.38	51.0	0.073	9.5
n	12	12	12	12	12	12
Mean	7.58±0.38	1.45±0.16	44.19±16.63	50.3±10.73	0.073±0.02	10.03±2.57

Table 2: The pharmacokinetic parameters of orally administered 500 mg Metronidazole in type II diabetic patients.

Diabetic Patients	C _{max} µg/ml	T _{max} hr	AUC _{last} µg hr ml ⁻¹	AUC _{total} µg hr ml ⁻¹	K _{elim} hr ⁻¹	T _{1/2} hr
D1	5.85	2.0	83.14	89.21	0.053	13.02
D2	6.21	2.0	46.31	54.60	0.034	19.91
D3	5.9	2.0	67.84	72.61	0.054	19.66
D4	4.8	2.0	67.46	72.88	0.051	13.58
D5	5.2	1.5	69.98	79.34	0.041	16.56
D6	5.67	1.5	62.84	68.49	0.050	13.97
D7	5.6	1.5	64.78	70.13	0.051	13.59
D8	5.86	2.0	62.37	64.63	0.067	10.30
D9	5.9	2.0	71.44	77.7	0.051	13.48
D10	5.29	1.5	62.69	68.13	0.050	13.90
D 11	6.75	1.5	66.79	72.88	0.060	15.69
D12	4.5	1.5	64.99	70.66	0.040	13.91
n	12	12	12	12	12	12
Mean	5.63±0.38	1.75±0.16	65.89±9.24	71.77±9.26	0.05±0.01	14.8±3.03

Table 3: The pharmacokinetic parameters of orally administered 500 mg Metronidazole in type II diabetic patients compared to healthy subjects.

Pharmacokinetic parameters	Healthy subjects	Diabetic patients
C _{max} (µg/ml)	7.58 ± 0.38	5.63 ± 0.42*
T _{max} (hr)	1.45 ± 0.16	1.75 ± 0.01*
AUC _{last} (µg hr ml ⁻¹)	44.19 ± 16.63	65.89 ± 9.24*
AUC _{total} (µg hr ml ⁻¹)	50.30 ± 10.73	71.77 ± 9.26*
K _{elim} (hr ⁻¹)	0.073 ± 0.02	0.05 ± 0.01*
T _{1/2} (hr)	10.03 ± 2.57	14.42 ± 3.03*

Values are presented as mean ± SD; * significantly different compared to healthy subjects (P<0.05).

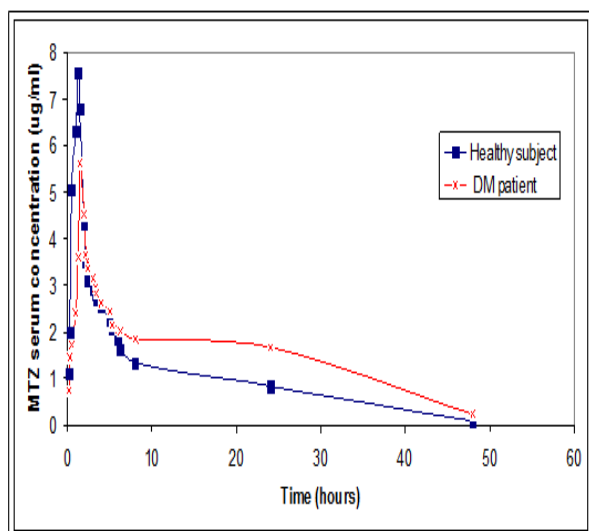


Figure 1: Cp-time profile of Metronidazole after 500 mg single oral dose in healthy subjects and patients with type II diabetes mellitus.

Discussion

The present study showed that the pharmacokinetics of the metronidazole following administration of a single 500 mg oral dose are different in patients with type II diabetes than in healthy subjects. Patients with type II diabetes exhibit significant decrease in C_{max} and AUC compared in healthy volunteers. The significant differences in metronidazole pharmacokinetic parameters between patients with type II diabetes and healthy volunteers indicates that the presence of diabetes had notable effects on the rate (T_{max} , K_{elim}) or extent (AUC, C_{max}) of absorption or elimination of metronidazole (K_{elim} , $T_{1/2}$) of the drug. Recent evidence suggests that hyperglycemia resulting from poor glycemic control may contribute to disturbances in gastric function in patients with diabetes ⁽¹²⁾. Other physiological changes associated with type 2 diabetes (such as alteration in renal and hepatic function and plasma protein binding) ^(12,13) would be expected to alter the pharmacokinetics of metronidazole, because metronidazole was completely absorbed after oral intake, less than 20% bound to plasma protein and extensively metabolized in the liver ⁽¹⁴⁾. Twenty to 30% of diabetics develop abnormal gastric motility, resulting in disordered gastric emptying or gastroparesis ⁽⁴⁾. Although the etiology of altered gastric motility remains obscure, many factors appear to be important including poorly controlled diabetes, and others ⁽¹⁵⁾. Absorption of many orally administered drugs may or may not be affected by the presence of diabetes; the extent of absorption of metoclopramide administered orally to diabetic patient with gastroparesis fell

within the range of values reported in healthy subjects ^(4,5). Meanwhile, absorption of tolazamide was 26% slower in diabetic patients with asymptomatic autonomic neuropathy than in healthy subjects ⁽⁴⁾, and a 26% decrease in the extent of absorption of orally administered ampicillin was reported compared with non-diabetics controls ⁽¹⁶⁾. Therefore, it appears that diabetes can influence the gastrointestinal absorption of drugs, but the extent of influence of the disease on drug absorption may depend on the severity, duration and type of the disease ⁽¹⁷⁾. Uncontrolled diabetes may be associated with changes in drug metabolism. Diajani *et al.* demonstrated a reduced rate of conversion of phenacetin to acetaminophen, and its subsequent conjugation was also impaired ^(4,18). Once the diabetes was controlled with insulin, metabolism became normal and subsequent withdrawal of insulin once again impaired metabolism. The finding of Morison and Hawks worth support these findings; where glucuronidation was deficient in microsomes prepared from streptozotocin-induced diabetes in male rats, and this defect was eliminated by insulin treatment ^(18,19). The liver is the main site of metabolism, and this accounts for over 50% of the systemic clearance of metronidazole. The two principal pathways of metabolism of metronidazole are oxidation and glucuronides ⁽¹⁴⁾. Diabetes is associated with an increased frequency and variety of hepatic histopathologic lesions. The most common lesion seen is an increase in liver glycogen demonstrated both at autopsy and in biopsy material. This accumulation of glycogen produces a clear appearance in the cytoplasm and vacuolization of the hepatocyte nuclei and has been reported in up to 80% of both type I and type II diabetic patients. Increased glycogen infiltration appears to be an associated consequence of exogenous insulin, resulting in hepatocyte accumulation of glycogen. This accumulation results in hepatomegaly, and could theoretically result in impaired hepatic elimination of some drugs. The metabolism of caffeine is decreased in diabetic patient while the clearance of paracetamol is decreased in diabetic patients type II ⁽¹⁷⁾. These evidences indicate that diabetes may affect biotransformation of metronidazole, which may alter the plasma levels and lead to increase its $T_{1/2}$. Also, this may explain why K_{elim} of orally administered metronidazole in diabetic patients less than in normal subjects. In conclusion, the pharmacokinetic parameters of metronidazole absorption after oral administration were altered in diabetic patients compared to healthy

subjects, and calculation of doses in this situation may be considered.

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References

- 1- Yu DT, Peterson JF, Seger DL, Gerth WC, Bates DW. Frequency of potential azole drug-drug interactions and consequences of potential fluconazole drug interactions. *Pharmacoepidemiol Drug Saf* 2005; 14:755-767.
- 2- White JR, Campbell RK. Dangerous and common drug interaction in patients with diabetes mellitus. *Endocrinol Metab Clin North Am* 2000; 29:789-802.
- 3- Kim YC, Lee A, Lee JH, Lee I, Lee DC, Kim SH, Kim SG, Lee M. Pharmacokinetics of theophylline in diabetes mellitus rats: Induction of CYP1A2 and CYP2E1 on 1,3-dimethyluric acid formation. *Eur J Pharm Sci* 2005; 26:114-123.
- 4- Gwilt PR, Nahhas RR, Tracewell WG. The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clin Pharmacokinet* 1991; 20: 477-490.
- 5- Jing M, Christopher K, Karen JL, Michael H. Diabetic Gastroparesis: Diagnosis and Management. *Gastroenterology* 2009; 69(8):971-986.
- 6- O'Connel ME, Awni WM, Goodman M, O'Melikian AP, *et al.* Bioavailability and disposition of metoclopramide after single- and multiple-dose administration in diabetic patients with gastroparesis. *J Clin Pharmacol* 1987; 27:610-614.
- 7- Chung M, Kourides I, Canovatchel W, Sutfin T, Messig M, Chaiken RL. Pharmacokinetics and pharmacodynamics of extended-release glipizide GITS compared with immediate-release glipizide in patients with type II diabetes mellitus. *J Clin Pharmacol* 2002; 42(6):651-657.
- 8- Della-Coletta AA, Eller MG. The bioavailability of tolazamide in diabetic patients and healthy subjects. *Pharmacol Res* 1988; 5:174.
- 9- Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, *et al.* Diagnosis and treatment of diabetic foot infections. *Plast Reconstr Surg* 2006; 117:212S-238S.
- 10- Emami J, Ghassami N, Hamishehkar H. A Rapid and sensitive HPLC method for the analysis of metronidazole in human plasma: Application to single dose pharmacokinetic and bioequivalence studies. *DARU* 2006; 14(1):15-20.
- 11- Mustapha KB, Odunola MT, Garba M, Obodozie O. Rapid, cost-effective liquid chromatographic method for the determination of metronidazole in biological fluids. *Afr J Biotechnol* 2006; 5(13):1188-119.
- 12- Daniyan MO, Omoruyi SI, Onyeji CO, Iwalewa EO, Obuotor EM. Pharmacokinetic changes of halofantrine in experimentally-induced diabetes mellitus following oral drug administration. *Afr J Biotechnol* 2008; 7(9):1226-1234.
- 13- Kim CY, Lee JH, Kim SH, Lee MG. Effect of CYP3A1 induction on clarithromycin pharmacokinetics in rats with diabetes mellitus. *Antimicrob Agents Chemother* 2005; 49(6):2528-2532.
- 14- Brunton LL, (Ed.), Goodman & Gilman's Pharmacology: The Pharmacological Basis of Therapeutics, 11th (Edn.), McGraw-Hill, New York, 2006.
- 15- Marangos MN, Athanasios T, Charles HS, Nightingale, *et al.* Absorption of ciprofloxacin in patients with diabetic gastroparesis. *Antimicrob Agents Chemother* 1995; 39(9):2161-2163.
- 16- Nicolian DP, Estein G. Therapeutic options for diabetic foot infections. *J Am Pediat Med Assoc* 2010; 100(1):52-63.
- 17- Preston RA, Epstein M. Effect of diabetes on cardiovascular drug metabolism. *Diabetes Care* 1999; 22(6):982-988.
- 18- Brenner AM, Bernasconi A, Garda HA. Effects of experimental diabetes on the fatty acid composition, molecular species of phosphatidyl-choline and physical properties of hepatic microsomal membranes. *Prostagland Leukotr Essen Fatty Acids* 2000; 6:167-176.
- 19- Lee DY, Lee MG, Shin HS, Lee I. Changes in omeprazole pharmacokinetics in rats with diabetes induced by alloxan or streptozotocin: Faster clearance of omeprazole due to induction of hepatic CYP1A2 and 3A1. *J Pharm Pharmaceut Sci* 2007; 10(4):420-433.