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Study of Urinary Excretion and Renal Clearance of Pioglitazone in Male Volunteers

MUHAMMAD ALIM[1,*], RAKHSHANDA NAWAZ[1], MUHAMMAD RAFIQUE ASI[2], MUHAMMAD SHAHID[1], SHAHZAD Z. IQBQL[1] and MATEEN ABBAS[1]

[1]Department of Chemistry and Biochemistry, University of Agriculture, 38040 Faisalabad, Pakistan
[2]Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan

*Corresponding author: Tel: +92 300 7605162 E-mail: prof_alim68@yahoo.com

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The urinary excretion and renal clearance of pioglitazone was investigated in 24 healthy male volunteers after oral administration of a single dose of 30 mg pioglitazone. Concentration of pioglitazone in plasma and urine was determined by high performance liquid chromatography (HPLC) and the concentration of creatinine by chemistry analyzer. In male volunteers the plasma concentration of pioglitazone was 0.576 µg/mL while the concentration of pioglitazone in urine was 0.277 µg/mL. Rate of urine flow (diuresis) recorded was 0.016 mL/min/kg. The renal clearance values of pioglitazone and endogenous creatinine were 0.012 and 0.672 mL/min/Kg, respectively. The ratio between the clearance of pioglitazone with the clearance of endogenous creatinine was 0.021. In urine of male volunteers, maximum concentration of pioglitazone excreted after 6 h was 0.463 µg/mL and cumulative amount of pioglitazone excreted was 150.77 µg.

Key Words: Pioglitazone, Renal clearance, Urinary excretion, Creatinine.

INTRODUCTION

Pioglitazone (±)-5-{[4-[2-(5-ethyl-2-pyridinyl)ethoxy] phenyl]methyl}-2, 4-thiazolidinedione is an oral antihyperglycemic agent used in the treatment of type 2 diabetes mellitus[1]. It acts primarily by decreasing insulin resistance in type 2 diabetes[2]. It is a peroxisome proliferator activated receptor (PPARγ) agonist that increases transcription of insulin responsive genes and thus increases insulin sensitivity[3-5].

Previous clinical trials in healthy volunteers have shown that pioglitazone is well absorbed after oral administration. It was first measurable in serum within 0.5 h. After absorption from the gastrointestinal tract, peak plasma concentrations were observed within 2 h. It is highly bound to plasma proteins (*ca.* 97 %), with a low tissue distribution and slow elimination (half-life *ca.* 9 h). It is extensively metabolized in the liver, with the majority excreted as inactive metabolites in the feces[6]. Following oral administration, *ca.* 15-30 % of the pioglitazone dose is recovered in the urine. Renal elimination of pioglitazone is negligible and the drug is excreted primarily as metabolites and their conjugates. It is presumed that most of the oral dose is excreted into the bile either unchanged or as metabolites and eliminated in the feces. Pioglitazone has an apparent clearance 5-7 L/h[7].

The environmental conditions under different geographical locations manipulate the genetic characters of the inhabitants living in that area. These geonetical influences are characterized by physiological and biochemical expression which are peculiar to the residents. These differences have important effects on pharmacokinetics of different drugs and eventually affect the response to the drugs[8]. These physiological and biochemical characteristics of a specie which influence the fate of the drugs have been explained by an original term geonetics[9]. Most of the drugs metabolizing genes show different activities in different populations which are often major determinants of variable drug exposure and response[10]. Moreover individuals can differ greatly in their inherent capacity to absorb, distribute, excrete and metabolize drugs. Therefore the present project was design to investigate the urinary excretion and renal clearance of pioglitazone as free drug in male volunteers under local conditions. This study along with other informations helps us to adjust the quantity and frequency of dose under our own ecological conditions.

EXPERIMENTAL

Volunteers: Healthy male volunteers (n = 24) were enrolled in the study. The age of the volunteers varied between 20-36 years and weight 54-74 kg. Those participants who fulfilling the inclusion criteria and provided voluntarily written consent on informed consent form were registered. The study was designed and conducted according to principles of good clinical practice (GCP) keeping in view the national legal requirements, the ICH harmonized tripartite guideline for GCP23 and the ethical principles laid down in the declaration of Helsinki.

TABLE-1
MEAN ± SE VALUES FOR AMOUNT OF PIOGLITAZONE EXCRETED IN URINE OF NORMAL MALE VOLUNTEERS AFTER DRUG ADMINISTRATION OF 30 mg TABLET

Time (h)	Amount (µg)	Percentage of drug	Cumulative amount (µg)	Cumulative (%)	Rate of excretion (µg/min/Kg)
1	3.70 ± 1.60	0.012 ± 0.005	3.70 ± 1.60	0.012 ± 0.005	0.001 ± 0.0005
2	15.50 ± 4.45	0.052 ± 0.015	19.19 ± 5.51	0.064 ± 0.018	0.005 ± 0.001
4	35.86 ± 6.26	0.120 ± 0.021	55.05 ± 9.98	0.184 ± 0.033	0.005 ± 0.001
6	55.51 ± 10.14	0.185 ± 0.034	110.56 ± 16.89	0.369 ± 0.056	0.008 ± 0.001
8	23.44 ± 5.55	0.078 ± 0.019	134.01 ± 20.03	0.447 ± 0.067	0.003 ± 0.001
10	13.99 ± 4.67	0.047 ± 0.016	148.00 ± 23.13	0.493 ± 0.077	0.002 ± 0.001
12	2.77 ± 1.38	0.009 ± 0.005	150.77 ± 23.52	0.503 ± 0.078	0.001 ± 0.0002

Drug administration: The drug pioglitazone commercially known Piozer® in the dosage form of oral tablets 30 mg each, manufactured by Hilton Pharma, Karachi, Pakistan was given to each volunteer with 250 mL of water.

Sample collection: Before drug administration, blank urine and plasma samples were collected from each volunteer. Following drug administration, blood and urine samples were collected at pre-determined time intervals. The samples were kept frozen in deep freezer at ≤ -20 ºC until further analyses.

Analysis: Both in plasma and urine samples, the concentration of pioglitazone was measured by high performance liquid chromatography as described by Zhang *et al.*[11], with some modifications. The modifications included the change in mobile phase ratio and pH and the use of internal standard. Limits of detection (LOD) and limits of quantification (LOQ) were evaluated from the signal-to-noise ratio. For plasma samples limits of detection obtained was 25 ng/mL and limits of quantification was 55 ng/mL. For urine samples the limits of detection obtained was 50 ng/mL and limits of quantification was 120 ng/mL. In plasma the average recovery of the assay was more than 79 % while in urine average recovery of the assay was more than 70 %. Extraction of pioglitazone in urine samples was carried out according to procedure described by Yamashita *et al.*[12].

The concentration of endogenous creatinine in the plasma and urine samples was determined by the method described by Bonsnes and Taussky[13]. Chemistry analyzer was used for the determination of creatinine using a creatinine kit by Jaffe reaction.

Calculation: The HPLC acquisition software (Class LC-10) was used for the qualitative and quantitative determination of pioglitazone. Other calculations and graphs were made using Microsoft Excel. The renal clearance of endogenous creatinine was used for the estimation of glomerular filtration rate (GFR). Renal clearance was calculated as given by Swenson[14]. Influence of urine pH, rate of urine flow and plasma drug concentration on the renal clearance of pioglitazone was examined by regression/correlation analysis. All data are reported as the mean ± SE as described by Steel *et al.*[15].

Safety: The study was conducted in accordance with good clinical practice guidelines (GCPG). According to the protocol approved by Ethics Committee, the safety examination of volunteers was recorded on case record form by doctors. All volunteers completed the study without referring any abnormality.

RESULTS AND DISCUSSION

Urinary excretion: In urine of male volunteers after oral administration of 30 mg pioglitazone, maximum concentration 0.463 µg/mL was observed after 6 h and then this concentration declined with time (Table-1). Maximum excretion of pioglitazone was observed 55.51 µg after 6 h and minimum 2.77 µg after 12 h. Cumulative amount of pioglitazone excreted in urine of male volunteers was 150.77 µg. The average per cent dose of pioglitazone excreted in urine was 0.012 % after 1 h, 0.185 % after 6 h. The minimum value for pioglitazone excretion was 0.009 % after 12 h post administration of drug. After 12 h of drug administration, the total drug excreted in urine was 0.503 % (Fig. 1). Average rate of excretion of pioglitazone was 0.001 µg/min/Kg at 1.0 h, 0.008 µg/min/Kg at 6 h and 0.001 µg/min/Kg at 12 h of drug administration in urine of healthy male volunteers.

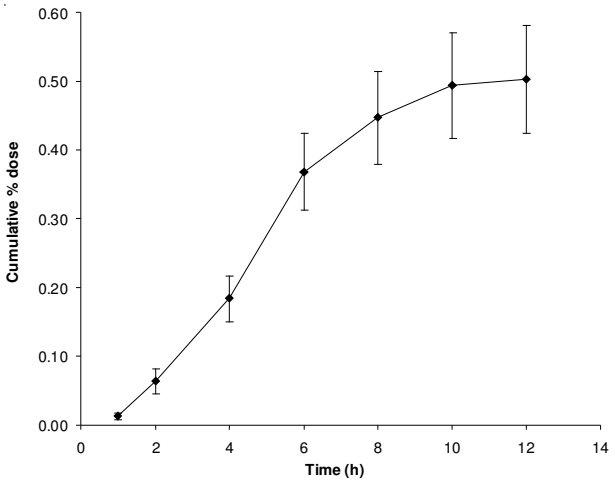

Fig. 1. Mean ± SE cumulative percentage of dose excreted in urine of 24 healthy male volunteers at various time intervals following oral dose of 30 mg pioglitazone tablet

Renal clearance: In the present study, in male volunteers the average ± SE value of 4 observations in 4 experimental periods for the rate of urine flow (diuresis) recorded in human male volunteers was 0.016 mL/min/kg (Table-2). The values of urine and blood pH are critical in biodisposition of drugs including their excretion in urine. The average value of urine pH in the present study was 5.29. The plasma concentration of pioglitazone was 0.576 µg/mL while the concentration of pioglitazone in urine was 0.277 µg/mL. The renal clearance values of pioglitazone and endogenous creatinine were 0.012

TABLE-2
MEAN ± SE VALUES FOR THE RENAL CLEARANCE OF ENDOGENOUS CREATININE AND PIOGLITAZONE FOLLOWING ORAL ADMINISTRATION OF 30 mg PIOGLITAZONE IN HUMAN MALE VOLUNTEERS

Parameters and units		Mean ± SE
Diuresis (mL/min/Kg)		0.016 ± 0.001
pH	Blood	7.43 ± 0.02
	Urine	5.29 ± 0.09
Creatinine	Plasma conc. (μg/mL)	9.26 ± 0.20
	Urine conc. (μg/mL)	374.75 ± 25.77
	Renal clearance (mL/min/Kg)	0.672 ± 0.046
Pioglitazone	Plasma conc. (μg/mL)	0.576 ± 0.045
	Urine conc. (μg/mL)	0.277 ± 0.043
	Renal clearance (mL/min/Kg)	0.012 ± 0.003
Clearance ratio	$Cl_{Pioglitazone}/Cl_{Creatinine}$	0.021 ± 0.006

and 0.672 mL/min/Kg, respectively. The ratio between the clearance of pioglitazone with the clearance of endogenous creatinine was 0.021.

The effects of plasma concentration of pioglitazone, rate of urine flow and urine pH on renal clearance of pioglitazone are shown in Figs. 2-4.

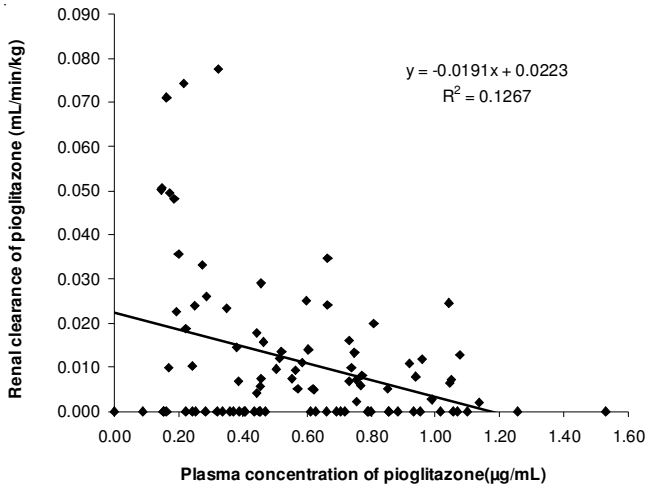

Fig. 2. Relationship between plasma concentration and renal clearance of pioglitazone after oral dose of 30 mg tablet in male volunteers

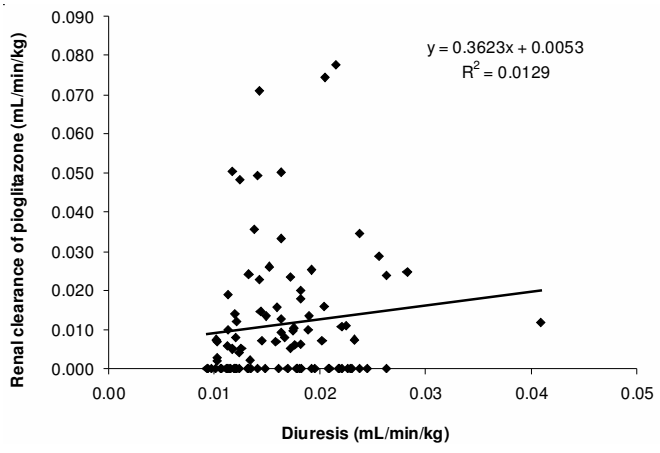

Fig. 3. Relationship between diuresis and renal clearance of pioglitazone after oral dose of 30 mg tablet in male volunteers

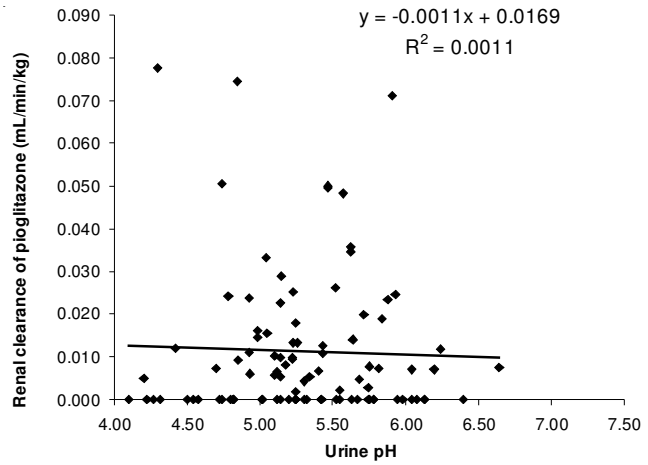

Fig. 4. Relationship between pH of urine and renal clearance of pioglitazone after oral dose of 30 mg tablet in male volunteers

In male volunteers after 12 h of drug administration, the total drug excreted in urine was 0.503 %. Average rate of excretion of pioglitazone was 0.001 μg/min/Kg at 1 h, 0.008 μg/min/Kg at 6 h and 0.001 μg/min/Kg at 12 h of drug administration. This indicates that in male volunteers only a small fraction of the administered dose of pioglitazone appears in the urine unchanged within 12 h. After oral administration, pioglitazone is excreted primarily as metabolites and their conjugates[16]. Identification of these metabolites and their routes of excretion may support to clarify the mechanism of pioglitazone clearance[17]. Renal elimination of pioglitazone is negligible and about 15-30 % of the dose along with metabolites is recovered in the urine[16,18]. Most of the oral dose is excreted into the bile either unchanged or as metabolites and eliminated in the feces[6].

Lower values of the renal clearance than the GFR indicate that the excretion of the drug through kidneys involves glomerular filtration and extensive renal tubular back diffusion or reabsorption[19]. The differences in the renal clearance values were attributed to environmental influences on glomerular filtration rate, blood composition and the pH of blood and urine. This is because previous studies demonstrated that environment has influence on the glomerular filtration. This is the reason that glomerular filtration rate under subtropical conditions is lower than in the indwellers of temperate environments[20]. The glomerular filtration rate also shows significant differences between the two, summer and winter seasons that affects the urinary excretion of drugs[21].

In the present study in male volunteers a significant positive correlation exists between diuresis and pioglitazone clearance (Table-3). Almost no considerable correlation was observed between urine pH and renal clearance of pioglitazone. However, negative correlation was observed between the plasma

TABLE-3
CORRELATION/REGRESSION OUTPUT:
Y = RENAL CLEARANCE OF DRUG WHEN

	x = Drug	x = Diuresis	x = Urine pH
Intercept	0.022	0.005	0.017
Slope	-0.019	0.362	-0.001
Correlation	-0.356	0.114	-0.033
r^2	0.127	0.013	0.001

plasma concentrations of pioglitazone with the renal clearance of pioglitazone. Much literature reviewed to compare the findings of the undertaken study but due to lack of studies worldwide on this aspect it was not possible to do it.

Conclusion

In the present study urinary excretion and renal clearance of pioglitazone in male volunteers were calculated. A significant positive correlation was exists between diuresis and pioglitazone clearance. Almost no considerable correlation was observed between urine pH and renal clearance of pioglitazone. However, negative correlation was observed between the plasma concentrations of pioglitazone with the renal clearance of pioglitazone.

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