Brief Communication

Biokinetic study of pioglitazone in female volunteers by a validated high performance liquid chromatography method

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Pioglitazone is an oral anti-hyperglycemic agent, which is used in the treatment of type 2 diabetes mellitus.[1] It is a member of "thiazolidinediones," which are the first drugs to address the basic problem of insulin resistance in patients with type 2 diabetes. It is the selective ligand of the nuclear transcription factor peroxisome-proliferator-activated receptor (PPARγ).[2] The environmental conditions under various geographical locations influence the genetical characters of the population living in that area. These genetical influences are characterized by physiological and biochemical manifestations, which are peculiar to the population. These differences have significant influences on bio-disposition, pharmacokinetics, and elimination of various drugs, and ultimately affect the response to the drugs. There is limited literature on the biokinetics/pharmacokinetics of pioglitazone in Pakistani volunteers. Therefore, the present project was design to develop a sensitive and precise analytical method for determining pioglitazone, and to evaluate pharmacokinetic parameters in human beings. This study will help us to adjust the quantity and frequency of pioglitazone dose under local conditions.

The study was carried out from March 2008 to April 2008 after the ethical approval from the Advance Studies and Research Board (ASRB), University of Agriculture, Faisalabad, Pakistan. Female volunteers (n=12), fulfilling the inclusion criteria and provided voluntarily written consent on an Informed Consent Form were registered. The sampling was conducted at the Center for Clinical Studies on Drugs, Independent Medical College, Faisalabad, and analysis was completed with the collaboration of Pesticide Chemistry Laboratory, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. The study was designed and conducted according to principles of good clinical practice, keeping in view the national legal requirements. The International Conference On Harmonisation (ICH) Harmonized Tripartite Guideline[3] for good clinical practice, and the ethical principles laid down in the Declaration of Helsinki[4] were followed. The concentration of pioglitazone in blood samples was determined by high performance liquid chromatography (HPLC) as described by Zhang et al[5] with some modifications. The modifications included the use of an internal standard, change in pH, and different mobile phase ratio. After oral administration of one tablet of pioglitazone hydrochloride (30 mg) by each volunteer, blood samples were collected at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10, 12, 24, and 36 hours in pre-heparinized centrifuge tubes 10 mL Vacuette® Griener Bio-One, Kremsmunster, Austria. A blank sample was also collected before medication. Tubes containing blood samples were protected from direct light and kept in an ice-chilled container until centrifuged. To separate the plasma, blood samples were centrifuged for 10 minutes at approximately 4000 rpm. After separation, the plasma fraction was transferred to a labeled glass tube and kept frozen in a deep freezer at ≤-20°C until analysis. For the calibration curve, standard stock solutions (100 µg/mL) of each pioglitazone (99.2%) and internal standard piroxicam (98.7%) were prepared by dissolving in dimethyl sulphoxide and methanol. Working standard solutions were prepared by the appropriate dilutions of the above-mentioned standard stock solutions. A series of standard solutions containing both the drug and the internal standard were also prepared. The stock and working solutions were stored in the dark under refrigeration. For HPLC, analysis samples were prepared in 10 mL glass tubes. In a tube, 1.0 mL plasma sample, 50 µL of internal standard (20 µg/mL), and 250 µL of 0.1 M dipotassium hydrogen phosphate was vortexed on a vortex mixer (Scientific Industries, Inc., New York, USA) for 30 seconds. Then, 5 mL ethyl acetate was added into the tube. This mixture was again vortexed for 3 minutes and centrifuged at 2130 x g (Shanghai Allcan Medical Co. Ltd, Shanghai, China) for 5 minutes. After centrifugation, 4 mL of organic layer was removed and evaporated to dryness under a stream of nitrogen at 45°C in a water bath. The residue was redissolved in 150 µL mobile phase; afterwards, 20 µL was directly injected onto the HPLC column. Shimadzu HPLC system (LC-10A) equipped with a fixed wavelength UV-Vis detector (Model SPD 10A, Shimadzu Corporation, Kyoto, Japan), column oven CTO 10A, liquid pump (LC-10AS), and acquisition software (Class LC-10) were used for the qualitative and quantitative determination of pioglitazone. The analytical column used to achieve chromatographic separation was a stainless steel (C18) column, Discovery Supelco, Cat. No. 568523 (25cm x 4.6mm, 5 µm) (Bellefonte, USA), protected by a guard column of the same material. All the chromatographic analysis was carried out at 30°C. The compounds were separated isocratically with a mobile phase consisting of acetonitrile and (0.1 M) ammonium acetate (41:59). Before use, the mobile phase was filtered by passing through a 0.45 µm membrane filter (Millipore, Bedford, Massachusetts, USA) and was sonicated through sonicator (Cole Palmer, Bunker Court, USA)

for 10 minutes. A constant flow rate of 1.0 mL/min was maintained. The effluent was monitored at a wavelength of 269 nm. The method was validated to demonstrate its linearity, accuracy, and repeatability. On the basis of plasma concentration versus time data, the pharmacokinetic parameters were determined using the PC-Computer Program, APO, MWPHARM version 3.02, a MEDIWARE product, Groningen, Holland. The plasma concentration of pioglitazone was measured by HPLC in the plasma samples. In all the samples collected at 36 hours, no drug was quantified. Therefore, the data has been calculated on the basis of last detectable concentration time. An HPLC chromatogram is shown in **Figure 1**, and average pharmacokinetic parameters of each volunteer are presented in **Table 1**. For computation and analysis of the drug plasma concentrations versus time data and the graphics, Microsoft Excel 7.0 was used. All data are reported as the mean ± SE. The limit of detection (25 ng/mL) and the limit of quantitation (55 ng/mL) of this method for different parameters indicates that it is a rapid and reliable method for the determination of pioglitazone in human plasma. Furthermore, the statistical evaluation of the proposed method led to the excellency of this method. In this method the pH of the mobile phase is a very important factor. A very small change in pH disturbed the retention time to a very large extent. Therefore, pH was kept at 4.10 ± 0.01 and was adjusted with the help of acetic acid (1 M).

Following oral administration of 30 mg pioglitazone by 12 female volunteers, the maximum concentration (Cmax) 0.96 µg/mL was achieved at a peak concentration time (tmax) of 2.35 hours. Average values of area under the plasma concentration-time curve (AUC) 8.13, volume of distribution 35.86 L (0.62 ± 0.15 L/kg), and elimination half-life of pioglitazone h.µg/mL, and 8.83 hours. Both differences and similarities are present in the values of different calculated parameters and the values present in the literature. These similarities and minor differences may be attributed to various genetic and environmental factors.

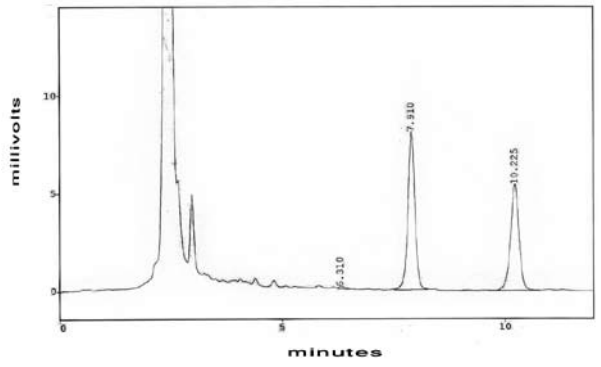

Figure 1 - A high performance liquid chromatography chromatogram of pioglitazone (RT=7.91) and internal standard (RT=10.22) in plasma.

Table 1 - Pharmacokinetic parameters of pioglitazone after an oral dose of 30 mg tablet to human female volunteers.

Parameters	Average	± SE
Area under the curve (AUC) (h.µg/mL)	8.13	1.22
AUC polyexponential (t=24)	7.33	1.09
AUC trapezoidal rule (t=24)	7.22	1.16
Clearance (L/h)	4.37	0.52
Volume of distribution comp.1 (L)	10.66	1.38
Volume of distribution steady state (L)	28.01	7.12
Volume of distribution (L)	35.86	8.81
Half-life phase 1 (h)	0.84	0.22
Half-life phase 2 (h)	6.23	1.39
Rate constant k10 (L/h)	0.51	0.11
Rate constant k12 (L/h)	1.93	1.36
Rate constant k21 (L/h)	0.87	0.45
Mean residence time (h)	9.35	1.35
Absorption rate constant (L/h)	0.82	0.19
Absorption half-life (h)	1.39	0.26
Lag-time (h)	0.56	0.15
Time to peak (tmax, h)	2.35	0.27
Peak concentration (Cmax, µg/mL)	0.96	0.12

comp - compartment, t - time, tmax - maximum time

In conclusion, we validated a simple, specific and sensitive HPLC method for the determination of pioglitazone in human plasma. The values of the calculated parameters in this study will support adjusting the dose of pioglitazone in the indigenous population.

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