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Renal Clearance and Urinary Excretion of Roxithromycin in Healthy Adult Female Subjects

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ABSTRACT

Objective: *In current study, influence of environmental situation and genetic variations on renal clearance and urinary excretion of roxithromycin was determined in local healthy adult female subjects. Roxithromycin is a second-generation macrolide widely used in human clinics for different infectious diseases.*

Methods: *For experimentation, ten healthy adult female were selected. Roxithromycin 300 mg (Rulide®) was given to each volunteer. Blank samples each for urine and blood was collected prior administration of roxithromycin and after that blood samples were collected at 1,2, 4 and 6 hours and urine samples were collected at 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours post medication. Blood samples were centrifuged to separate plasma. A high performance liquid chromatography (HPLC) method was used to find out the concentration of drug in urine and plasma samples. In plasma and urine concentration of drug and endogenous creatinine were used to calculate renal clearance and urinary excretion of drug.*

Results: *In present study the renal clearance of roxithromycin was 0.08 ± 0.01 ml/min/kg. Mean ± SE value for clearance ratio between drug clearance and creatinine clearance was 0.04 ± 0.00 which indicates reabsorption (back diffusion) of roxithromycin. The cumulative percentage of oral dose of 300 mg tablet of roxithromycin excreted through urine during 48 hours was 12.87 ± 1.31 percent.*

Conclusion: *Renal clearance and urinary excretion observed in present study variates from previous studies conducted in other geographical regions under different environmental conditions. Variations in present study may be due to genetic variations. It is concluded from the study that renal clearance and urinary excretion study is required under indigenous conditions.*

Keywords: Genetic variation, Urinary excretion, Renal clearance, Macrolide, HPLC, Endogenous

INTRODUCTION

Pakistan and most of the other developing countries import their raw material and finished form of drugs for use in animals and human health programs. Drugs are developed and tested through clinical and preclinical trials in drug exporting countries prior to their dispatch for export. The environmental conditions and genetic make-up of man and animals are different in drug exporting and importing countries for most of the cases. In many studies it has been depicted that pharmacokinetic behavior, renal clearance and urinary excretion data of the researched drugs showed dissimilar values under local environmental conditions as compared to the values given in the literature of the drug

provided by the manufacturer [1]. These variations depict the influence of environmental conditions on the genetics that alter the physiological indices which eventually affect the pharmacokinetics and fate of the drug in a population [2]. Extensive research in human models has revealed that the bio-disposition of some drugs in local geonetical conditions is dissimilar to the records of dis-position elsewhere. So, the rational approach for the designing of optimal dosage is based upon the pharmacokinetic data determined in local indigenous environment in which the drug is employed and the type of species [3]. Pharmacokinetic and pharmacological behavior of drugs is also affected by the concomitant use of other drugs, in such cases there is a need to rationalize the individual's therapy [4].

Macrolides are obtained from the Streptomycin. Roxithromycin belongs to macrolides antibiotic. It is an ether oxime semi-synthetic derivative of erythromycin A. This derivative of erythromycin A has improved acid stability and pharmacokinetic properties. Roxithromycin synthetically prepared by modification of ketone at C-9. It is bactericidal drug at high and bacteriostatic drug at low concentrations. Roxithromycin inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit of bacteria. Roxithromycin has been recurrently used for the cure of a variety of infections such as bacterial infection linked with stomach and intestinal ulcer [5]. Roxithromycin because of their anti-inflammatory effects are used for respiratory Infections, asthma, cystic fibrosis, urinary, soft tissue and orodental infections. Keeping in view the preceding lines, renal clearance and urinary excretion of roxithromycin was planned to investigate in healthy adult female subjects [6].

MATERIALS AND METHODS

Renal clearance and urinary excretion of roxithromycin were investigated in 10 healthy adult female subjects in local population. The experimental procedure for the present study is given below.

Ethical consideration

All volunteers were informed about the purpose of study, frequency of sampling and possible side effects of drug and a written permission with each volunteer was made before the start of research.

Experimental subjects

Ten healthy female subjects were chosen to accomplish the research at the Department of Physiology and Pharmacology, University of Agriculture, Faisalabad.

Selection criteria

Age of volunteers was in the range of 25-30 years, weight and height of all volunteers were measured, volunteers were not having any disease and physical abnormality, physical examination of each volunteer was made, laboratory investigations and clinical history of all volunteers were made to declare them healthy persons, volunteers were not taking any medication.

Methodology

Drug administration

Rulide® (Roxithromycin), 300 mg tablet, Sanofi Aventis Pvt. Ltd., Karachi. The selected female volunteers were given roxithromycin 300 mg orally.

Collection of blood samples

For the collection of blood samples, disposable syringes were used to vacate blood directly from the forearm of each volunteer. The blood samples were collected in heparinized plastic centrifuge tubes. Prior to the drug administration, a control blood sample was collected from all volunteers. Roxithromycin tablets were given orally to each volunteer and the samples were collected at 1, 3, 4 and 6 hours post medication. The pH of each blood sample was recorded with the help of electric pH meter. The blood samples were centrifuged at 4000 rpm for 5 minutes. Plasma was separated and preserved at -20°C until analysis.

Collection of urine samples

Prior to the drug administration, a control urine sample was collected from each volunteer. Then Roxithromycin tablets were given orally and the urine samples were collected at time intervals of 1, 2, 4, 6, 8, 10, 12, 24, 36, and 48

hours after drug administration. The volume and pH of each urine sample was checked. 20 ml sample was stored at -20°C until analysis.

Roxithromycin analysis

Roxithromycin concentrations in the samples were analyzed by using High Performance Liquid Chromatography (HPLC) method [7,8]. All oxime compounds are detected and quantified easily by reverse phase HPLC with UV detectors [9].

Chemical and solvents

Heparin sodium salt, HPLC Grade Chemicals, Roxithromycin standard, Acetonitrile, Methanol, Deionized water, all the solvents were of analytical grade, Dichloromethane, Sodium chloride, Phosphate buffer.

Instrumentation

Analytical Balance (Sartorius, Germany), Centrifugation Machine (MSE, Micro Centaur, Sanyo UK), Filtration Assembly (0.45 µm), Micropipettes: 10 µL to 1000 µL (Oxford, Ireland), Sonication Apparatus (Oqawaseiki Co,Japan), Liquid Chromatographic Pump (Syknm S1122), System Controller Unit (Syknm), UV Visible Detector (Syknm S3210), Sample Injector (Syknm S5111 Valve Bracket).

Chromatographic conditions

Mobile Phase: Acetonitrile, Deionized water and Methanol

Flow Rate: 0.5 ml/min

Wavelength: 300 nm

Injection Volume: 20 µL

Column: C-18 thermo- hypersil (250 × 4.6 nm × 5 µm)

Temperature: 30°C

Detector: UV-Visible Detector

Preparation of mobile phase

The mobile phase was consisted of Acetonitrile, double distilled water and Methanol. The water for mobile phase was prepared by double glass distillation. The ratio of Methanol, Acetonitrile and water was 45:30:25 respectively. After mixing the solvents, mobile phase was passed through filtration assembly, having the filter paper size of 0.45 µm Whatsman (Schleicher and Schuell 12.5 mm). Then, the filtered mobile phase was sonicated for 15 minutes to remove any air bubbles.

Preparation of stock solution and standards

The standard stock solution of roxithromycin was prepared by dissolving 3.2 mg of roxithromycin standard in 1 mL of distilled water to make a final concentration of 3200 µg/mL. The working solutions of the concentrations of 1, 1.5, 2, 3, 5 and 6 µg/mL were made by appropriate dilution of the stock solution as needed to construct the standard calibration curve. Aliquots of these working standard solutions were stored at -20°C. These solutions were filtered through Whatman membrane filters of 0.45 mm pore size (25 mm filter) and 20 µL was injected into HPLC for analysis. Calibration graph was prepared by using peak area verses concentrations of working solutions.

Preparation of plasma samples

250 µL of plasma samples were taken and transferred to 2 ml of polyethylene vial in which 1 ml of acetonitrile and acetic acid was already added. The samples were centrifuged at 4000 rpm for 1 minute. The sample contents were separated into aqueous and organic layers. The aqueous layer was discarded and the organic phase was transferred to 2 ml glass vials. The solvent was evaporated to dryness at 40°C under stream of nitrogen. The residue was re-dissolved in 250 µl of mobile phase of which 20 µL was taken and injected in chromatographic column for roxithromycin analysis.

Preparation of urine samples

Frozen urine was allowed to thaw at room temperature. Dichloromethane (5 ml), sodium chloride (0.25 g) and 0.5 M phosphate buffer (pH 8.0) (500 µL) were added to 1.0 ml of urine in a screw capped tube. After shaking with a vortex mixer for 10 min and centrifugation at 4000 rpm for 15 min, the aqueous layer was discarded. The remaining organic phase was transferred into a new glass tube and evaporated with a vacuum evaporator at 40°C. The residue was reconstituted with 200 µl of the mobile phase and 20 µl were injected into HPLC apparatus.

Standard curve

These working standard solutions were analyzed through HPLC. The calibration curves were constructed by plotting the concentration versus peak area data on a graph. The curves were linear over the range of 1 to 6 ug/ml for roxithromycin (Figure 1).

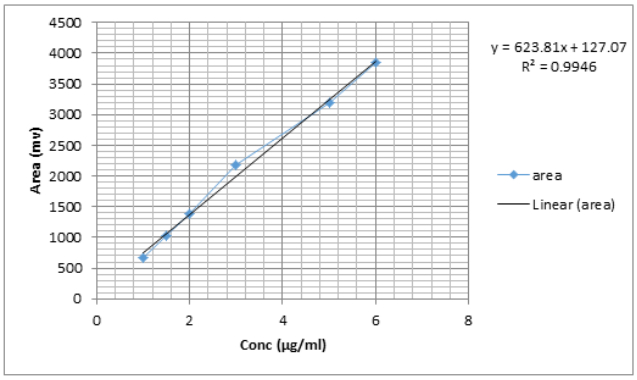

Figure 1: Standard curve of roxithromycin

Determination of roxithromycin in plasma and urine samples

The concentration of roxithromycin in samples was determined by the following regression equation:

$Y = bx + a$

Where, y=Peak area for unknown concentration, a=Intercept, b=Slope of the regression line and x=Concentration of drug.

RESULTS AND DISCUSSION

The mean ± SE data related to the renal clearance of endogenous creatinine and Roxithromycin after oral dose of 300 mg tablets in 10 healthy female has been calculated. Blood and urine samples were collected at specific time intervals. The values of diuresis, pH of blood and urine samples, concentration of endogenous creatinine and roxithromycin in blood and urine samples, renal clearance of endogenous creatinine and roxithromycin and clearance ratio between roxithromycin and endogenous creatinine has been measured and presented in Table 1.

Correlation between pH of urine and clearance ratio

There was a non-significant ($p>0.05$) positive correlation ($r=0.06$) between pH of urine and roxithromycin clearance as shown in Figure 2.

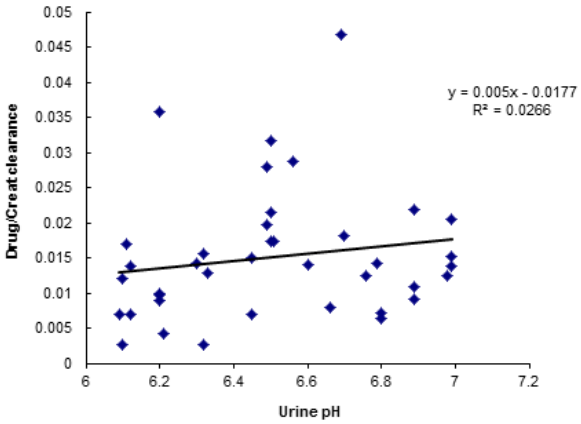

Figure 2: Effect of urine pH on renal clearance of roxithromycin after single oral dose of roxithromycin 300 mg in 10 female subjects. Each data point shows one of the 40 observations in 10 experiments, each comprise of 4 experimental periods

Correlation between diuresis and clearance ratio

There was a significant ($p>0.05$) positive correlation ($r=0.028$) between rate of urine flow and roxithromycin renal clearance as shown in Figure 3.

Table 1: Mean ± SE renal clearance of endogenous creatinine and roxithromycin in 10 healthy adult female subjects after single oral dose of 300 mg tablet

Animal No.	Body weight (kg)	Diuresis ml/min/kg	pH		Creatinine conc. mg/ml		Drug conc. mg/ml		Renal clearance ml/min/kg		Ratio rox/ creat
			Blood	Urine	Plasma	Urine	Plasma	Urine	Creat	roxithro	
1	46.00	0.04	7.45	6.13	11.00	420	8.76	21.64	1.58	0.10	0.06
2	45.00	0.04	7.46	6.13	9.67	435	8.57	13.00	1.86	0.06	0.03
3	45.00	0.04	7.40	6.44	11.00	460	8.72	13.51	1.88	0.07	0.04
4	46.00	0.05	7.47	6.50	11.10	544	8.22	19.97	2.66	0.13	0.05
5	48.00	0.04	7.43	6.62	10.10	510	8.20	13.92	1.99	0.07	0.03
6	49.00	0.04	7.45	6.63	10.90	420	8.66	14.00	1.59	0.07	0.04
7	49.00	0.03	7.46	6.71	8.70	420	8.34	15.03	1.38	0.05	0.04
8	52.00	0.03	7.47	6.65	12.00	458	8.50	14.59	1.08	0.05	0.04
9	50.00	0.04	7.47	6.57	11.70	600	9.20	14.86	1.86	0.06	0.03
10	50.00	0.05	7.43	6.38	10.60	630	8.64	16.71	2.80	0.09	0.03
Mean	**48**	**0.04**	**7.45**	**6.48**	**10.68**	**489**	**8.58**	**15.72**	**1.87**	**0.08**	**0.04**
± SE	**0.77**	**0.00**	**0.01**	**0.07**	**0.32**	**14.62**	**0.07**	**1.02**	**0.15**	**0.01**	**0**

Each data point is mean of four observations in four experimental periods.

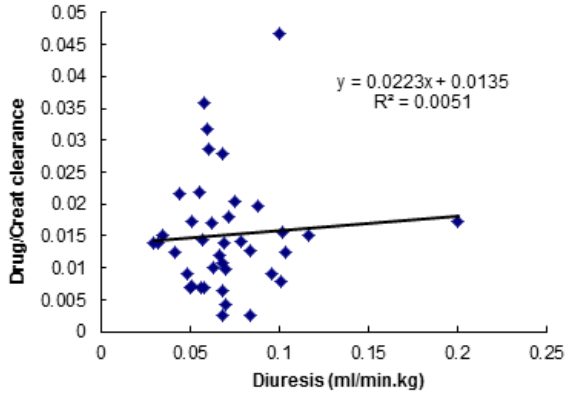

Figure 3: Effect of diuresis on renal clearance of roxithromycin after single oral dose of roxithromycin 400 mg in 10 female subjects. Each data point shows one of the 40 observations in 10 experiments, each comprise of 4 experimental periods

Plasma concentration of roxithromycin versus clearance ratio

There was a significant ($p<0.05$) negative correlation ($r=-0.193$) between plasma concentration of roxithromycin and renal clearance as shown in Figure 4.

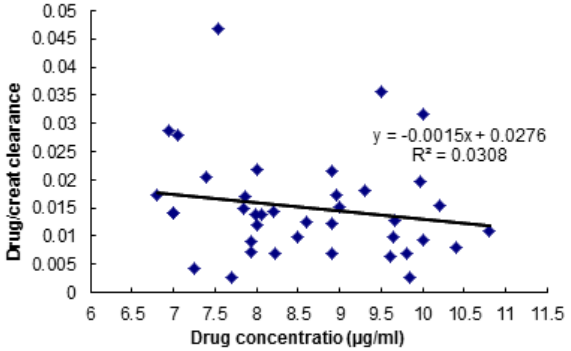

Figure 4: Effect of plasma concentration on renal clearance of roxithromycin after single oral dose of roxithromycin 300 mg in 10 healthy female subjects. Each data point shows one of the 40 observations in 10 experiments, each comprise of 4 experimental periods

Urinary excretion

The urinary excretion of roxithromycin was measured in 10 healthy human female volunteers. The results of urinary excretion are expressed in terms of amount excreted in mg (Table 2 and Figure 5), percentage dose of roxithromycin excreted (Figure 6) and cumulative percent dose excreted (Figure 7).

Table 2: Mean ± SE of dose excreted (mg) of roxithromycin in urine of healthy adult female subjects after single oral dose of 400 mg tablet

Dose excreted in mg									
Time (min)									
	60	120	240	360	480	600	720	1440	2880
1	6.82	2.2	5.62	3.52	1.17	9.1	10.2	4.66	8.24
2	2.61	0.54	0.64	0.26	0.79	5.85	14.2	0.8	6.2
3	0.20	0.75	2.04	3.06	0.90	2.65	16.4	5.99	5.5

4	3.10	3.19	1.30	5.7	1.67	6.49	20.6	4.68	8.47
5	2.17	2.04	1.13	1.05	0.97	4.35	9.03	7.36	3.01
6	1.10	1.10	1.44	2.12	1.69	4.49	9.73	8.39	6.24
7	1.23	1.93	1.18	0.94	0.73	2.18	8.99	4.96	4.73
8	1.33	1.38	1.13	0.94	1.18	3.56	6.97	9.42	7.49
9	1.94	1.23	0.91	1.89	1.06	3.47	13.2	7.34	7.04
10	2.11	3.61	6.03	0.83	2.5	5.11	17.2	7.5	8.21
Mean	2.26	1.80	2.14	2.04	1.27	4.72	12.6	6.13	6.52
SD	2.04	0.87	1.58	1.83	0.37	2.25	4.62	2.66	1.84
±SE	0.64	0.27	0.50	0.58	0.11	0.71	1.46	0.84	0.58

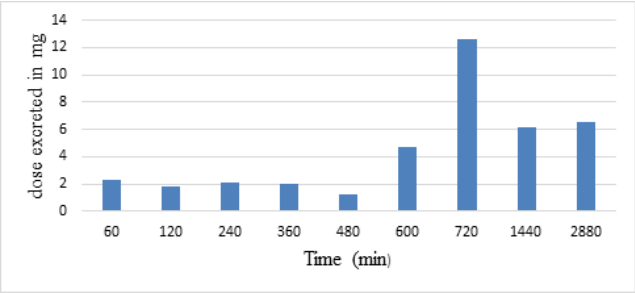

Figure 5: Mean ± SE value of dose (mg) excreted of roxithromycin in urine of healthy female volunteers after single oral dose 300 mg tablet

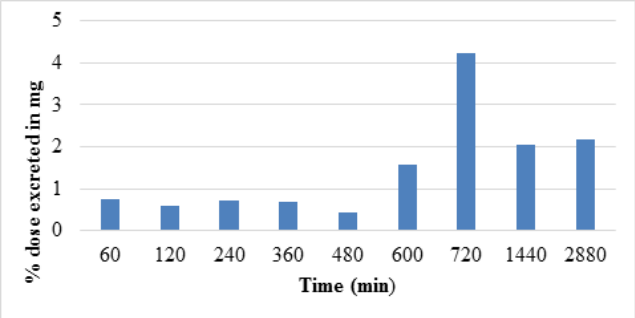

Figure 6: Mean ± SE value of percentage dose excreted of roxithromycin in urine of healthy female volunteers after single oral dose 300 mg tablet

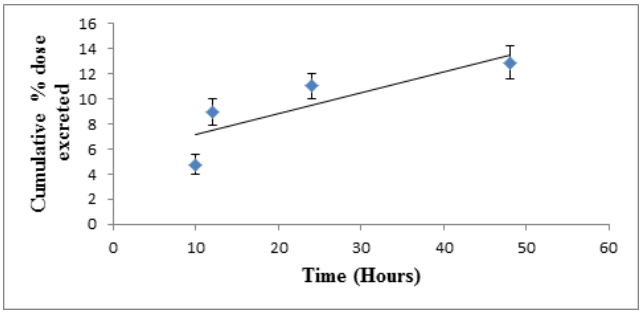

Figure 7: Mean ± SE cumulative percent of dose excreted at 10, 12, 24, and 48 hrs after single oral dose of roxithromycin 300 mg tablet

CONCLUSION

The present investigations on renal clearance and urinary excretion of roxithromycin in healthy adult female volunteers have inferred that renal handling of roxithromycin shows that besides glomerular filtration, passive diffusion of drug is also involved at kidney tubular level. Lower urinary excretion of roxithromycin also reflects the extra renal route for its excretion, may be biliary excretion. The difference between renal clearance and urinary excretion parameters of present study and those reported in the literature clearly indicates that these parameters of a drug should be determined in the species and environment in which the drug to be employed.

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The analyses were performed by using an HPLC chromatograph in Department of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. Department has provided instruments and chemicals. Sample collection was author's research.

REFERENCES

[1] Min, T.H., et al., *Experimental Parasitology,* **2007.** 115(4): p. 387-392.
[2] Javed. I., et al., *Pakistan Veterinary Journal,* **2006.** 26(1): p. 1-8.
[3] Javed, I., et al., *Turkish Journal Veterinary and Animal Sciences,* **2009.** 33(4): p. 300-310.
[4] Alina, Z., et al., *Journal of Biomedical Sciences,* **2016.** 6(1): p. 1-6.
[5] Ogawa, N., et al., *European Journal of Pharmacology,* **2003.** 474(2-3): p. 273-281.
[6] Ferrara, G., et al., *Respiratory Medicine,* **2005.** 99(1): p. 1-10.
[7] Macek, J., Ptacek, P, and Klima, J., *Journal of Chromatography. B, Biomedical Sciences and Applications,* **1999.** 723(1-2): p. 233-238.
[8] Lahane, S.B., Deokate, U.A, and Ahire, S.K., *International Journal of Pharmaceutical Sciences Review and Research,* **2014.** 26(2): p. 256-261.
[9] Ahmad, S., et al., *ARC Journal of Pharmaceutical Sciences,* **2016.** 2(1): p. 37-57.