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Renal Clearance of Endogenous Creatinine and Doxycycline in Male Volunteers

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Doxycycline is a broad spectrum antibiotic that inhibits protein synthesis in bacteria. Renal clearance of the drug and that of endogenous creatinine was determined by oral administration of 2×100 mg doxycycline in healthy male volunteers. Before drug administration control samples and after drug administration blood and urine samples were collected, at predetermined time periods. The samples were assayed for endogenous creatinine by spectrophotometric methods and doxycycline by microbiological assay. Mean±SE values for the blood and urine pH mean were 7.81±0.030 and 6.13±0.187, respectively. The rate of urine flow (diuresis) was 0.056 ± 0.011 ml/min/kg and creatinine was cleared at rate of 1.109±0.386 ml/min/kg of body weight. Average±SE value for doxycycline clearance was 0.49±0.056 ml/min/kg and mean±SE value for clearance ratio was 1.046±0.366. A significant positive correlation between diuresis and renal clearance of doxycycline indicate the back diffusion or reabsorption of the drug.

Key words: Renal clearance, creatinine, doxycycline, male volunteers

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Introduction

Doxycycline is 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-Octahydro-3,5,10,12,12a pentahydroxy-6-methyl-1,11-dioxo-2-Naphthacene carboxamide. The molecular formula of doxycycline monohydrate is $C_{22}H_{24}N_2O_8.H_2O$ and a molecular weight of 462.46. Doxycycline is a broad-spectrum semi-synthetic antibiotic, derived from oxytetracycline and is available as doxycycline monohydrate; doxycycline hyclate; doxycycline hydrochloride hemiethanolate hemihydrate and doxycycline calcium for oral administration. Doxycycline has a high degree of lipoid solubility and a low affinity for calcium binding. It is highly stable in normal human serum. It acts bacteriostatically by binding with ribosome and thus inhibiting the bacterial protein synthesis. It is used against organisms like ***Escherichia coli***, ***Mycoplasma pneumoniae***, ***Streptococcus pneumoniae***, ***Haemophilus influenzae*** and ***Enterococcus faecalis***. It is considered the drug of choice for atypical pneumonia, anthrax, rickettsial diseases, cholera, urinary tract infection and for sexually transmitted diseases (Natalie and Cunha, 1995). The drug is mainly eliminated through the intestinal tract and in the urine (about 40% in 72 h).

Therefore studies of mechanisms involved in the renal handling are of practical importance. The rate of glomerular filtration can be determined by measuring the renal clearance of substances that are neither secreted nor reabsorbed by the tubules and exert no effect on renal function. In all mammalian species, creatinine is freely filtrated in the same concentration as in plasma, so renal clearance of creatinine is a widely used parameter for the assessment of kidney function (Bowers and Wong, 1980). The creatinine clearance value is determined from the creatinine concentration in serum and pooled 24 h urine samples. A direct measure would be the renal clearance of creatinine measured during each period of urine collection. As creatinine is predominantly excreted via glomerular filtration, so regarded as direct measure of glomerular filtration. The present research project was designed to study the renal clearance of creatinine and doxycycline in male volunteers under local environmental conditions. As the biochemical parameters recorded under indigenous conditions differs from that of foreign counterpart due to Geometric difference (Nawaz and Shah, 1985; Nawaz ***et al.***, 1984; Nawaz, 1994)

Materials and Methods

Drug and administration and sampling

Renal clearance of endogenous creatinine and doxycycline was determined in 8 healthy adult male volunteers following 2×100 mg tablets of doxycycline. The physical parameters include average body weight, age and heights of volunteers were noted. The blood samples were drawn in heparinized centrifuge tubes at 0, 30, 60, 90, 120, 150, 180 and 240 min after the drug administration. Urine samples were taken at 0, 30, 60, 90, 120, 150 and 180 min. The blood samples were centrifuged at 4,000 rpm for 15 min to separate plasma and stored at -20°C for further analysis.

Analytical technique

For the estimation of glomerular filtration rate (GFR), the endogenous creatinine renal clearance was measured in plasma and urine samples spectrophotometrically by the method of

Bonsnes and Taussky (1945).To 0.5 ml of plasma, 4 ml of N/12 H_2SO_4 and 0.5 ml of 10% sodium wulfamate was added and centrifuged at 4000 rmp for 15 min. To 3 ml of supernatant, 2 ml of fresh alkaline picrate were added and absorbance was noted at 515 nm wavelength. First for samples of urine were diluted to 1:50 and in 3 ml of each dilution, 2 ml of freshly prepared alkaline picrate was added and absorbance was noted.

Microbiological assay

Doxycycline concentration in plasma and urine was determined by microbiological assay according to the method of Arret ***et al.*** (1971) by using ***streptococcus fecalus*** as test organism. Paper disks were placed on surface of medium containing nutrient agar and incubate at 37°C for 18 hours and the zones of inhibition were measured. Different parameters were calculated as:

$$\text{(a)}\quad \text{Diuresis (D)} = \frac{\text{Urine volume in collection period (ml)}}{\text{Time (min.)} \times \text{Body wt. (kg)}}$$

(b) Creatinine concentration:

$$\text{Absorbance} \times \text{standard factor X dilution factor}$$

(C) Doxycycline concentration: $Y = a - bx$

Where

X = zone of inhibition (mm)

Y = Concentration of drug ($\mu g\ ml^{-1}$)

$$\text{(d)}\quad \text{Renal clearance (Cl)} = \frac{\text{Concentration of substance in urine (Uc)} \times \text{diuresis (D)}}{\text{Concentration of substance in plasma (Pc)}}$$

$$\text{(e)}\quad \text{Clearance Ratio} = \frac{\text{Renal clearance of drug}}{\text{Renal clearance of creatinine}}$$

Results and Discussion

The results showing mean ± SE of the experimental periods of pH of blood and urine, diuresis, plasma and urine concentration of creatinine, doxycycline concentration, doxycycline clearance and clearance ratio have been presented in Table 1 and graphically shown in different figures.

In this study the average ± SE value of pH in blood was 7.81 ± 0.03. This value is almost equal to the previously reported value of blood pH 7.73 ± 0.02 in male volunteers. Average±SE value of pH in urine was 6.13±0.187 which is comparable to the value 5.85 ± 0.08 reported earlier on male volunteers. The average±SE value for the rate of urine flow (diuresis) recorded in human male volunteers was 0.056±0.014 ml/min/kg. This value is higher than that reported earlier in winter season which was 0.04±0.004 ml/min/kg (Naseem, 1999). This difference is due to

Table 1: Average data of renal clearance of endogenous creatinine and doxycycline in male volunteers after oral dose of 100×2 mg (200) tablets

					Concentration μg ml^{-1}						
	Body weight	Diuresis ml/	pH		Creatinine		Drug		Renal clearance		Ratio
Volunteers	kg.	min. Kg.	Urine	Blood	Plasma	Urine	Plasma	Urine	Creatinine	Drug	Cld/Cler
1	61	0.020	5.64	7.70	13.72	994.06	3.026	56.68	0.953	0.374	0.034
2	72	0.046	6.44	7.96	8.75	298.63	3.024	38.34	1.409	0.631	0.447
3	55	0.031	5.69	7.78	5.52	74.00	2.791	53.49	0.415	0.594	1.431
4	53	0.0503	6.70	7.78	8.59	115.20	2.790	23.06	1.034	0.415	0.401
5	75	0.027	5.64	7.86	8.55	943.60	3.390	59.47	1.318	0.473	0.358
6	78	0.041	6.14	7.77	9.78	45.29	2.157	30.55	0.189	0.580	3.068
7	62	0.100	7.00	7.78	8.43	234.83	2.060	39.14	2.590	0.19	1.9
8	60	0.087	5.81	7.90	9.63	418.48	3.156	37.08	1.777	0.67	1.022
Mean	64.5	0.056	6.13	7.81	9.152	435.80	2.89	42.22	1.109	0.49	1.046
±SE	3.300	0.011	0.187	0.030	0.802	144.92	0.166	4.923	0.386	0.056	0.366

environmental difference because in hot climate the evaporation reduces the urine flow during summer while lower environmental temperature increases the rate of urine during winter (Nawaz and Shah, 1984).

In the present study the average ± SE value of creatinine clearance in male volunteers was 1.109±0.297 ml/min/kg. It slightly differ than the values reported earlier for male volunteers i.e. 1.95±0.164 ml/min/kg (Yasin ***et al.***, 2001) and for female volunteers i.e. 1.13±0.07 ml (min/ml/kg) (Naseem, 1999).

The average ± SE value for the doxycycline concentration in plasma was 2.89 ± 0.166 μg/ml and the results were comparable to earlier study of (Taneja ***et al.***, 1974) in which the plasma drug concentration of earlier study in almost similar, so age and gender has no effect on bio-disposition of drug. That is why dose adjustment based on age and gender is not necessary as the peak serum concentration reached at the same time in all subjects after dosing (Saux ***et al.***, 1982).

The average±SE value for the renal clearance of doxycycline was 0.491±0.056 ml/min/kg. The present value is slightly lower than the renal clearance value 0.538 ml/min/kg body weight reported by (Taneja ***et al.***, 1974) due to difference of environment and temperature.

Average±SE value for renal clearance ratio was 1.046±0.35. From the data it is cleared that clearance of doxycycline was lower than the endogenous creatinine clearance or filtration clearance (GFR). This indicates that re-absorption or back diffusion take place (Taneja ***et al.***, 1974).

The studies indicate that the renal clearance of endogenous creatinine in male volunteers was lower than the values given in the literature. There is significant positive correlation between diuresis and doxycycline clearance (Fig. 1). As the rate of urine flow increases, rate of doxycycline clearance also increases. There is slightly significant positive correlation between pH of urine and doxycycline clearance (Fig. 2).This is because in acidic pH of urine drug becomes unionized and is reabsorbed and with increase in pH it becomes ionized hence renal clearance increases.

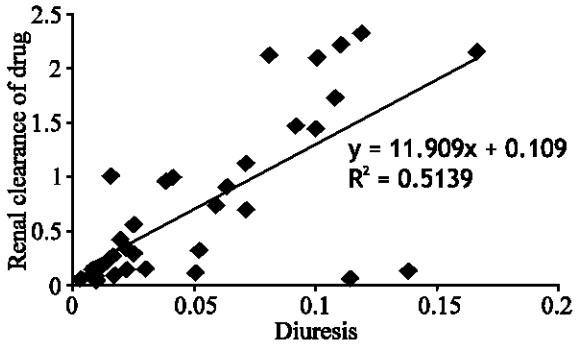

Fig. 1: Relationship between diuresis and renal clearance of drug

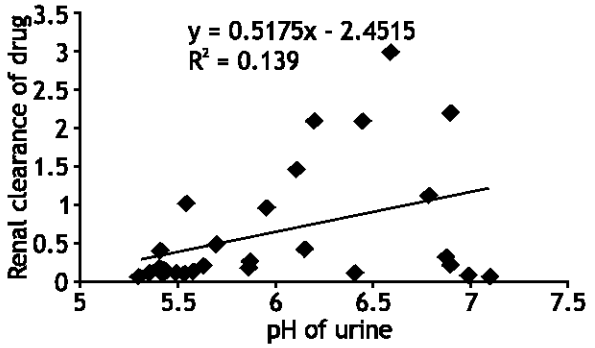

Fig. 2: Relationship between pH of urine and renal clearance of drug

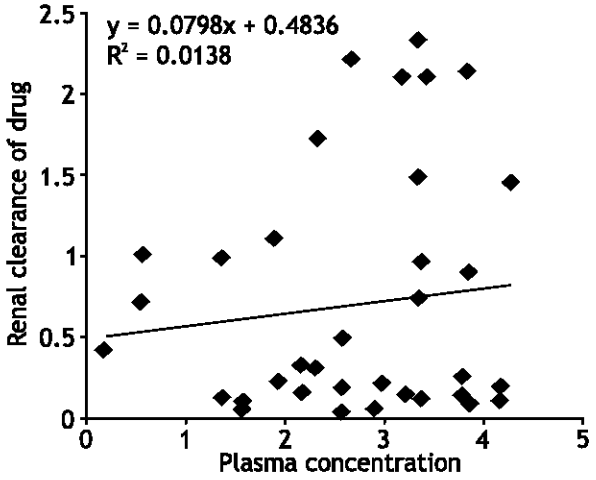

Fig. 3: Relationship between plasma concentration of drug and renal clearance of drug

The non-significant positive correlation between doxycycline and plasma concentration and renal clearance (Fig. 3) shows that when drug is retained in plasma, renal clearance decreases and vice versa. The lower values of renal clearance support the previous findings that there is a need to evaluate the imported drug under indigenous conditions for getting optimal therapeutic effects and to highlight the lacunae in our knowledge of the drug's pharmacodynamics in human body.

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