

Excretion of Trimethoprim Through Urine of Healthy Males

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ABSTRACT

Drug disposition investigated under indigenous conditions showed variations when compared with the values given in the literature. These variations emphasize the basis of indigenous investigations. Urinary excretion of trimethoprim was investigated in eight healthy human (male) volunteers. The drug was given orally as single dose and the urine samples were collected at predetermined time intervals after the drug administration. The average pH, concentration and rate of excretion were 6.44 ± 0.164 , 11.676 ± 2.77 $\mu\text{g/mL}$ and 0.185 ± 0.397 $\mu\text{g/min/kg}$, respectively. The cumulative per cent dose of trimethoprim excreted in urine of healthy male after 12 h following its oral administration (160 mg) was 9.667 ± 1.081 .

Key Words: Trimethoprim; Urinary excretion; Spectrophotometer

INTRODUCTION

Trimethoprim [2, 4-diamino-5-(3, 4, 5-trimethoxybenzyl)-pyrimidine] an antibacterial agent, was discovered in the Wellcome Research Laboratories in Tuckahoe, New York (Glawisching *et al.*, 1972). Septran DS (Wellcome Research Laboratories) has two constituents' trimethoprim (160 mg) and sulphamethoxazole (800 mg). The antibacterial activity of trimethoprim and sulphonamide results from their action in two steps on enzymatic pathway for the synthesis of tetrahydrofolic acid. The effect of trimethoprim is due to specific inhibition of the bacterial dihydrofolic acid reductase, an enzyme that facilitates the reduction of dihydrofolic acid into tetrahydrofolic acid. The affinity of trimethoprim for this bacterial enzyme is approximately 50,000 times greater than corresponding mammalian's enzyme. The sulfonamide intervenes and obstructs the para-aminobenzoic acid (PABA), and the formation of dihydrofolate is also collectively inhibited. A combination of trimethoprim and sulfonamide is widely used resulting in a synergistic therapeutic effect. Antimicrobial activity of sulfonamides is potentiated by trimethoprim in a very low drug concentration. The

combination of these two antibacterial chemotherapeutic substances results in synergy and makes a bactericidal, a broad spectrum in activity (Blood *et al.*, 1983).

Commercially, trimethoprim and sulphonamide is marketed with different brands due to their vast use as potent synergistic antibacterial agent. The well known combinations are co-trimoxazole (trimethoprim & sulphamethoxazole), co-trimazine, trimethoprim and sulphadiazine and co-trifamole, trimethoprim and sulphamoxole. Trimethoprim is also used with sulphamethopyrazine and sulphametrole or sulphadimidine which are largely used as veterinary medicines practiced with sulphadoxine (Goodman & Gilman, 1996).

The developing countries like Pakistan are importing drugs for their human and veterinary health programmes. Pharmacokinetic behavior, renal clearance and urinary excretion of the drugs are different under different climatic conditions, socio-economic status, psychosomatic problems and environmental stress. Therefore, trials under indigenous conditions may differ as compared with the values given in the literature supplied by the product manufacturers (Nawaz & Shah, 1985). It necessitates conducting local studies of trimethoprim to know its partial profile, as excretion by the body or in other words accumulation in the tissues. These variations warrant depiction of therapeutic standards and dosage regimens on the basis of indigenous investigations. The present project was, therefore, planned to analyze the trimethoprim in urine of healthy males under indigenous environmental condition.

Table I. Absorbance of standard concentration of Trimethoprim in urine at 271 nm

Concentration ($\mu\text{g/mL}$)	Absorbance	Standard factor (conc./absorbance)
2	0.147	13.6
4	0.262	15.27
6	0.438	13.70
8	0.542	14.76
10	0.671	14.90
12	0.785	15.29
14	0.886	15.80
16	0.996	16.06
Average		14.92

MATERIALS AND METHODS

Drug. The drug is available commercially as Septran DS, Bactrim forte, Nicotrim forte and Cotran etc. in different formulations, the more commonly available are tablets containing 160 mg of trimethoprim and 800 mg

sulphamethoxazole for oral use. Septran DS manufactured by Glaxo Wellcome Karachi Ltd. was the original research product, therefore, preferably purchased for studying purpose.

Volunteers. Eight healthy male volunteers aged 21-24 years (mean age 22.6 years) and weight 55-80 kg (mean weight 61-5 kg) participated in this trial after their written willful consents. All volunteers were in good and sound health, no previous history of hypersensitivity to drug and any ailments or malfunctioning

During study volunteers, who suffered from allergic and problem of hypersensitivity, gastrointestinal distress, emetic or nausea were discontinued. These healthy beings were strictly monitored and no other medications were allowed to take one week before the beginning of study and during the study. Body weight, age, height, blood pressure and temperature of each volunteer were recorded (not shown).

Sampling procedure. The sampling was started on 15th of July 2002 in a very hot season while the temperature was between 40-45°C. Before drug administration urine was taken this sample was considered as control or blank for comparison. The urine samples of each volunteer were collected at 60, 120, 180, 240, 360, 480 and 720 min after the drug oral administration. The pH of all urine samples was determined with the highly precise and accurate pH meter. The collected samples were kept and stored at -20°C till the completion of whole study.

Drug analysis. Each urine sample was analyzed in triplicates. Drug was extracted from urine samples by chloroform. Then it was back extracted into aqueous media using 0.1N H₂SO₄ and its amount was determined at 271 nm spectrophotometrically (Clarke, 1974).

Standard curve. Stock solution was prepared by dissolving 100 mg trimethoprim in 100 mL of solution containing a few drops of 0.1 N H₂SO₄ (1000 µg/mL). Then 10 mL of this solution was diluted up to 100 mL by adding distilled water to get 100 µg/mL of stock solution.

From the stock solution containing 100 µg/mL trimethoprim, the standard solutions containing 2, 4, 6, 8, 10, 12, 14 and 16 µg /mL trimethoprim were made by making volume up to the comparable volume of urine.

For the analysis of trimethoprim, 1 mL from each stock solution was taken in test tubes and 0.5 mL 0.1 N NaOH was added. The contents were mixed thoroughly. After mixing, 6 mL chloroform was added to the mixture and mixed vigorously in stoppered test tubes for one minute. These contents were centrifuged at 2000 rpm for 10 minutes. The organic layer was separated and 3 mL of 0.1 N H₂SO₄ was added. The contents were again mixed vigorously for one minute and then centrifuged for 10 min at 2000 rpm. The aqueous layer was separated and the absorbance was taken at 271 nm with spectrophotometer (Hitachi U 2001 spectrophotometer, 12-1250-01(1995) Japan).

The absorbances of standard solutions are shown in

Table II. pH of the urine voided and trimethoprim excretion by healthy males after oral administration

Time (min)	pH (Mean±SE)	Trimethoprim excretion (µg/mL)
60	6.05±0.233	13.365±3.574
120	6.30±0.211	15.090±4.655
180	6.34±0.148	13.223±4.235
240	6.50±0.121	7.777±0.663
360	6.58±0.179	9.250±1.707
480	6.67±0.141	13.088±3.059
720	6.63±0.118	9.938±1.507
Average	6.44±0.164	11.676±2.77

Table III. Rate and cumulative excretion of Trimethoprim ((µg/min/kg) in urine of healthy male after its oral administration (160 mg)

Time (min)	Rate of excretion (Mean±SE)	Cumulative TMP excretion (Mean±SE)
60	0.390±0.098	0.866±0.206
120	0.234±0.060	1.871±0.418
180	0.253±0.040	3.284±0.672
240	0.178±0.032	4.893±0.797
360	0.121±0.024	6.556±0.994
480	0.084±0.014	8.010±1.116
720	0.036±0.010	9.667±1.081
Average	0.185±0.040	-

the Table I. The standard factor was calculated by dividing the concentration with absorbance. The values of absorbance were plotted against the concentration of trimethoprim (µg/mL) (Fig. 1). Urine samples taken at different time intervals after oral administration of the drug were quantitatively analyzed for trimethoprim.

RESULTS AND DISCUSSION

The average pH, concentration and rate of excretion of drug overall in study are 6.44±0.164, 11.676±2.77 µg/mL and 0.185±0.040 µg/min/kg shown in Table II and III, respectively. A significant correlation between pH and rate of excretion was observed as depicted (Fig. 2). The value of R₂ indicates that there is negative significant correlation between pH of urine and rate of excretion. This means rate of excretion of trimethoprim increase with the decrease in pH of urine and vice versa.

The cumulative per cent of trimethoprim excreted in urine of healthy males at different time intervals after 160 mg drug administration is shown in Table III. The cumulative percentage of trimethoprim excreted in urine after 60 and 180 min was 0.866±0.206 and 3.824±0.672 ranging from 0.162 to 1.618 and 0.943 to 5.975% respectively. After 360 and 720 min of drug administration dose excreted were 6.556±0.994 and 9.667±1.081 ranging from 3.396 to 10.382 and 5.911 to 13.152%, respectively. Cumulative urinary excretion as per cent of dose excreted versus time (min) is shown in Fig. 3.

The total recovery of unchanged trimethoprim is

Fig. 1. Standard curve of trimethoprim in urine of healthy males

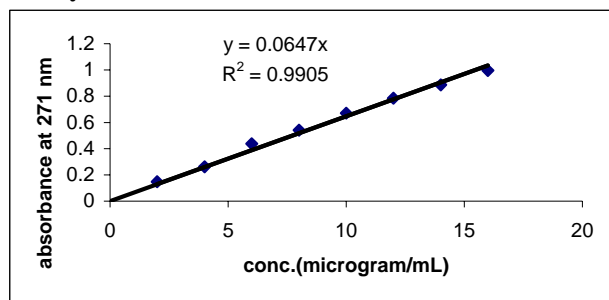


Fig. 2. Relationship between pH and rate of excretion of trimethoprim in healthy males

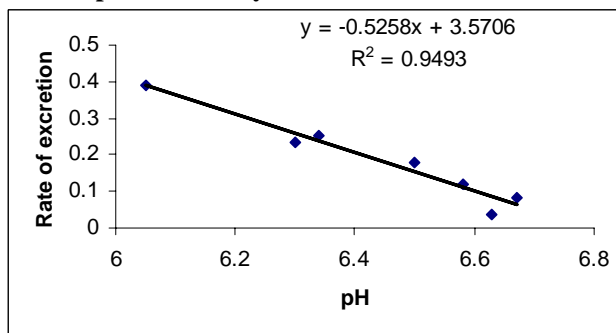
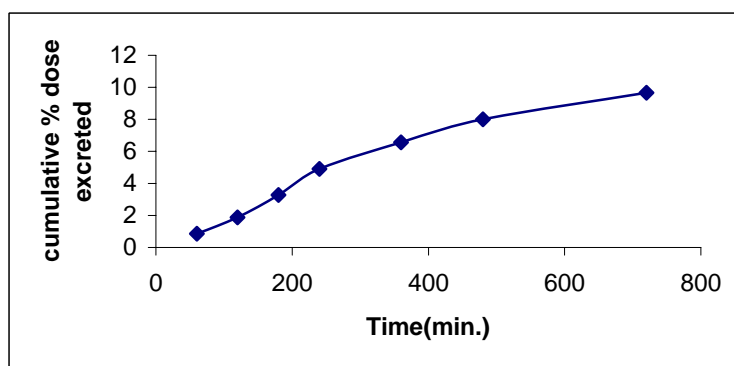


Fig. 3. % cumulative amount of trimethoprim excreted in the urine of healthy males after its oral administration (160 mg)



9.667% after oral dose of 160 mg tablet after 720 min. Less than 15% of the dose was excreted unchanged in urine of swine (Nielsen & Rasmussen, 1975). In another study, the total per cent amount excreted in urine of healthy males was 5% (Stachoska & Senczuk, 1987).

The difference is due to environmental effects and genetic characteristics that are manifested by the variation in the biochemical, physiological and pharmacological parameters. These differences may be due to variability in gender, fluctuation in urine pH and nutritional ingredients. Species variation may also effect the urinary excretion (Nawaz, 1994).

There is a contradiction in our values and foreign investigated values. This contradiction may be due to environmental factors, physiological differences, and genetic factors which effect chemical nature and pharmacokinetics of trimethoprim in our local environmental conditions.

CONCLUSION

This study suggests that local preclinical and comprehensive trials must be conducted before allowing a drug to be marketed for public health care; moreover, diet and nutrition play a significant role. Physicians should also

properly guide the patients about their intake because rate of excretion of drug depends upon quality, quantity and nature of food.

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