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Analysis of metabolites of trimethoprim in urine of human male volunteers

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Abstract

Trimethoprim is an antibacterial drug used to treat peoples with urinary tract infections. The metabolism of Trimethoprim, an antibacterial drug, was studied in urine of human male volunteers. Urine samples were collected at pre-determined time intervals. The concentration of Trimethoprim as free and total drug was analyzed by spectrophotometer at 271 nm and the metabolites were separated by paper chromatography. The average ± SE values for the amount of free and total Trimethoprim in urine of human male volunteers were found to be 4.949 ± 0.228 µg/mL and 6.749 ± 0.264 µg/mL respectively. The R_f values of free and metabolite of Trimethoprim in male volunteers were found to be 0.39 & 0.225, respectively. The method of analysis is precise, accurate, easy and reproducible.

Key words: Chromatography, Metabolites, Spectrophotometer, Trimethoprim, Urine.

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1. Introduction

The development of the anti-microbial agents to prevent and cure bacterial infections is one of the 20th century's major contributions to human longevity and quality of life [1]. Trimethoprim is an antibacterial drug used to treat people with urinary tract infections. The combination drug product Trimethoprim/ sulfamethoxazole (TMP/SMX) is used to treat a wide variety of bacterial infections and some infections due to parasites. Trimethoprim [2, 4-diamino-5-(3, 4, 5,-trimethoxibenzyl) pyrimidine] is an antibacterial agent, was discovered in the Welcome Research Laboratories in Tuckahoe, New York [2]. The principal metabolites of Trimethoprim are the 1- and 3-oxides, the 3'- and 4'-hydroxy and two N-oxide derivative of Trimethoprim. When administered together as sulfamethoxazole; Trimethoprim, neither sulfamethoxazole nor Trimethoprim affects the urinary excretion pattern of the other [3]. Trimethoprim is a dihydrofolate reductase inhibitor. It inhibits the conversion of bacterial dihydrofolic acid to tetrahydrofolic acid which is necessary for the synthesis of certain amino-acids, purines, thymidine and ultimately DNA synthesis. Trimethoprim is active against a wide range of aerobic Gram-negative & Gram positive organisms including strains of most enterobacteriaceae. For testing the antimicrobial activity of TMP the media should contain minimal amounts of thymine or thymidine. Minimum inhibitory concentrations for most susceptible organisms have been reported to range from 0.1 to 5.0µg/mL [4]. Trimethoprim produces the predictable adverse effects of an antifolate drug, especially megaloblastic anemia, leucopenia, and granulocytopenia. It may cause nausea and vomiting, drug fever, vasculities, renal damage, and central nervous system disturbances occasionally occur also [5].

Several studies have revealed that chemically and biologically determined urine concentration of drugs show differences when compared with values given in the literature. Hence the dosage regimen of drug should be different. Therefore, it is important to generate information about biodisposition and metabolism of Trimethoprim in local population and environment to establish our own therapeutic standards.

2. Material and Methods

2.1. Subjects

Ten healthy volunteers participated in this study were having average age 22.5 years, average weight 61.5 Kg and average height 5.48 feet. On the basis of physical examination and medical history it was determined that all volunteers were in good health. No other medications were permitted one weak prior to and during the study.

2.2. Drug and drug administration

The drug Trimethoprim commercially known Septran DS in the dosage form of oral tablets 160 mg each, manufactured by Glaxo Welcome Ltd., Karachi, Pakistan,

and were used. The sampling was done in the month of July, 2003.

2.3. Urine sample collection

After an overnight fast control urine samples were collected from all volunteers before drug administration. The volunteers were given one tablet each of Trimethoprim (Septran DS, 160mg) with a glass of water orally. The volunteers were allowed to take breakfast after one hour the following drug administration. The urine samples were taken at 0, 60, 120, 180, 240, 360,480 and 720 minutes after the administration of drug. The volume of urine voided during this period was noted and pH of each sample was recorded. A small volume of each urine sample was stored in plastics bottle and preserved in freezer at -20ºC for further analysis.

2.4. Extraction of drug without acid hydrolysis

For the extraction of drug, took 1mL of urine from each sample in triplicate and added 0.5mL of 0.1N NaOH. Then added 6mL Chloroform for the extraction of drug in organic layer and shake for 1 minute and centrifuged at 4500 rpm for 5 minutes. Then drug is back extracted with 3mL of 0.1N H_2SO_4 mixed for 1minute and centrifuged at 4500 rpm for 5 minutes, and noted the absorbance with spectrophotometer. (Hitachi-U 2001 Spectrophotometer Japan) at 271 nm [6].

2.5. Extraction with acid hydrolysis

Total amount (metabolites and free drug) of drug was determined because metabolites in the urine samples changed in to free Trimethoprim after acid hydrolysis. Took 1mL of urine sample from each sample in triplicate separately, add 1mL of 4N HCl in each sample and heated them on boiling water bath for 1hr., to convert the metabolites into free drug. Took them out from the boiling water bath, and repeat the same procedure as mentioned above.

2.6. Analysis of urine

Each urine sample was analyzed in triplicate using spectrophotometric method. The basis of this method is to record the absorbance of drug after its extraction from urine.

2.7. Quantitative analysis of metabolites of Trimethoprim by paper chromatography

Take 780 mL n-butanol, distilled H_2O 43.3 mL and 4.80g citric acid. Draw a horizontal line leaving 5 cm sheet behind it. Sheet 14x6 in buffered by dippining in a 5 % solution of citric acid + tri- sodium citrate drying for one hour. Then made 5 spots consisting standard solution, control sample and preserved sample of 6, 8 and 12 hours (free and total).The concentration of applied standard drug solution was 30 µL. Then placed the sheet in the chromatographic tank containing solvent, After 5 hours, sheet was taken out for examination under ultraviolet light and also with Ninhydrin spray, determine the R_f values by using the formula [6].

$$Rf = \frac{\text{Distance traveled by sample}}{\text{Distance traveled by solvent}}$$

2.8. Statistical analysis

The results were tabulated and statistical calculations are done according to standard method. The results are given as average ± SE [7].

3. Results and Discussion

The results showed the average ± SE values for diuresis 0.035 ± 0.008 & 0.049 ± 0.020 ml/min/Kg, pH (6.239±0.080) and rate of excretion of free and total Trimethoprim were and 0.065±0 .011 & 0.080 ± 0.019 µg/min/Kg for male respectively. The average ± SE value for the amount of free and total Trimethoprim (mg) in urine of male volunteers were 0.908 ± 0.185 mg & 1.502 ± 0.358 mg respectively. The study conducted by Babar [8] showed the amount of Trimethoprim excreted was 1.962±0.487 mg. This study showed that amount of total Trimethoprim excreted in urine is 11.19% while Nielson and Rasmussen [9] reported the 15% of the dose was excreted. The study conducted by Stachoska and Senezuk [10], showed the percentage dose excreted in urine was 5%. The results showed the average ± SE values for cumulative dose for the amount of free and total Trimethoprim were 6.457 ±0.795 & 6.926 ±0.795 mg for male volunteers respectively. While another study showed that cumulative amount of TMP excreted was 24.6mg [11]. The cumulative amount of TMP excreted was 18.6 mg observed in six human adult volunteers [12]. In the present study the amount of Trimethoprim excreted as free and metabolites in urine is ranged from 4.389 to 12.278 mg from which the amount of metabolites of Trimethoprim is ranged from 0.528 to 1.722 mg while remaining is the free form. The percent of dose excreted in urine over a 12-hour period following the intravenous administration of the first dose of 240 mg of trimethoprim and 1200 mg of sulfamethoxazole on day 1 ranged from 17% to 42.4% as free Trimethoprim [13]. There are some difference between the values of present study and foreign literature which is compared above. This difference may be due to variability in dose, gender variation, and fluctuation in urine pH, environmental conditions and nutritional ingredients. Species variation may also effect the urinary excretion [14]. The overall difference between the values of present study from the foreign literature may be due to difference in pH, low glomerular filtration rate, gender difference, age, temperature and time for the recovery of drug. Also the method applied in this study is not too sensitive.

4. Conclusion

The result of this study indicated that the pharmacokinetic study of Trimethoprim was found to be slightly different in local subjects. Moreover, the data also show differences when compared with foreign values. The study supports the need for comprehensive evaluation of drug under local environment to obtain pharmacokinetic parameters on which the rational dose regimens of drug could be prescribed.

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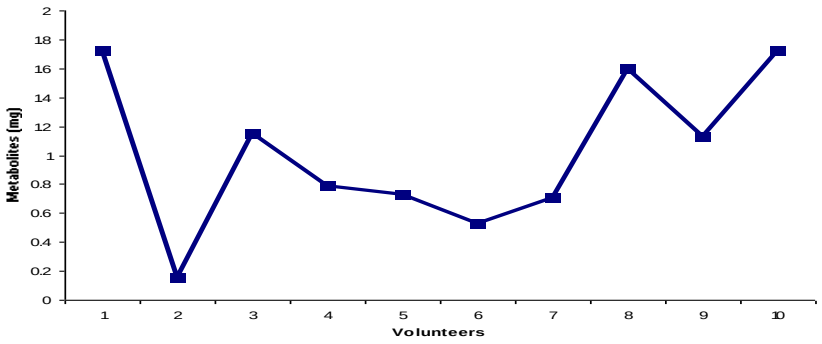

Fig. 1. Plot between amount of metabolite (mg) and volunteer.

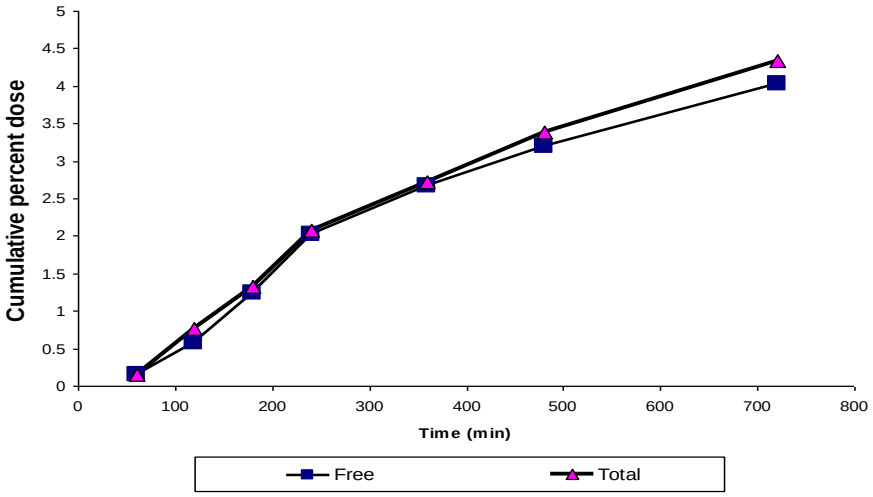

Fig. 2. Cumulative amount of Trimethoprim excreted as free and metabolites in urine of male volunteers after oral administration (160mg)

Table 1. The average amount of Trimethoprim excreted as free and total in urine of male volunteers

	Time (min)						
Drug form	60	120	180	240	360	480	720
Free	0.073	0.088	0.104	0.085	0.051	0.027	0.030
Total	0.081	0.100	0.116	0.087	0.052	0.029	0.035

Table 2. The average %age amount of Trimethoprim excreted as free and total in urine of male volunteers

	Time (min)						
Drug form	60	120	180	240	360	480	720
Free	0.172	0.431	0.701	0.781	0.622	0.473	0.895
Total	0.483	1.184	1.957	1.849	1.836	1.339	2.542

Table 3. Cumulative percentage dose of Trimethoprim excreted as free and total in urine of male volunteers

	Time (min)						
Drug form	60	120	180	240	360	480	720
Free	0.161	0.573	1.250	2.030	2.666	3.200	4.035
Total	0.144	0.768	1.330	2.066	2.728	3.389	4.329

Table 4. Amount of Trimethoprim excreted as free and metabolites in urine of male volunteers after oral administration (160mg)

Volunteers	A	B	C	D	E	F	G	H	I	J
Cumulative amount	7.567	12.425	10.693	7.440	5.931	5.306	5.557	7.385	5.521	6.710
After hydrolysis (mg)										
Cumulative amount	5.845	12.272	9.540	6.650	5.199	4.778	4.853	5.79 0	4.389	5.259
Before hydrolysis (mg)										
Metabolites (mg)	1.722	0.153	1.153	0.790	0.732	0.528	0.704	1.595	1.132	1.722

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