Pharmacokinetic Study of a New Derivative of Sulfamethoxazole

Authors: M. S. Iqbal[1], A. H. Khan[2], M. Saeed[3], M. Sher[4]

Affiliations:
[1] Deparment of Chemistry, Forman Christian College, Lahore, Pakistan
[2] Department of Pharmacy, Jinnah Hospital/Allama Iqbal Medical College, Lahore, Pakistan
[3] Department of Medicine, Jinnah Hospital/Allama Iqbal Medical College, Lahore, Pakistan
[4] Department of Chemistry, University of Sargodha, Sargodha, Pakistan

Key words
- pharmacokinetics
- salicylidine-sufamethoxazole-Zn(II)
- sulfamethoxazole
- zinc complex
- HPLC

Abstract

The study was aimed at determination of pharmacokinetic parameters of a previously synthesized salicylidine-sulfamethoxazole-Zn(II) monohydrate in normal humans. This new derivative of sulfamethoxazole was reported to be more active and less toxic than the parent drug by our group. 10 volunteers received a 200 mg dose of the drug orally. Blood samples were collected just before and after 0.16, 0.33, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 h of administration of the drug. The plasma samples were analyzed for sulfamethoxazole by a new validated high performance liquid chromatography method having a suitable limit of quantification. The dose of each drug was well tolerated without any adverse effect. The maximum plasma sulfamethoxazole concentration was 280 µg L^{-1} at a t_{max} 1.30 h. This suggests a rapid onset effect of the complex as compared with the parent drug. The plasma half-life, clearance, and volume of distribution of sulfamethoxazole from salicylidine-sulfamethoxazole-Zn(II) monohydrate were 1.64 h, 0.24 L h^{-1} and 0.57 L kg^{-1} respectively. The elimination of sulfamethoxazole followed the first order kinetics with $R^2 > 0.984$. The larger value of volume of distribution and clearance for the new derivative, as compared to that of the parent drug, show that the new derivative may exhibit prolonged antimicrobial effect with rapid clearance.

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Correspondence
M. S. Iqbal, PhD
Department of Chemistry
Forman Christian College
Ferozepur Road
Lahore 54600
Pakistan
Tel.: +92/300/4262 813
Fax: +92/42/9923 0703
saeediq50@hotmail.com

Introduction

The wider use of antibiotics in humans and animals and in areas other than the treatment and prophylaxis of disease have resulted in a serious problem of drug resistance. Various strategies have been worked out and tried to cope with the resistance problem and enhance the activity, or broaden the spectrum of drugs [1]. Preparation of different synthetic derivatives of antibacterials based on structure-activity relationship has been one of the best approaches. It has been demonstrated that metal Schiff base complexes of some antibiotics possess good potential as more effective and safe drugs [2,3]. Several metal complexes of sulfamethoxazole (smz) [4] and its derivatives have been reported in literature including the Schiff base derived from salicylaldehyde [3–5] but no attention has been paid to study biodistribution of such compounds in vivo. The present work reports the pharmacokinetic study of a new derivative of sulfamethoxazole, previously reported to be less toxic than the parent drug [3], by use of a new validated high performance liquid chromatography (HPLC) method.

Participants and Methods

Participants

10 healthy volunteers (5 male and 5 female), aged between 18–25 years, median age of 21 with 10 % of ideal weight and build, were selected. The participants were educated about the type of study; safety of medicine and possible undesirable effects etc. All the procedures followed were in accordance with the current revision of the Helsinki Declaration, and all the subjects used in this study gave informed consent.
The study was approved by the Ethics Committee of the University of Sargodha after reviewing the safety profile of the drug substances under investigation. The study was carried out at the affiliated hospital of the University under responsibility of a consultant physician at the hospital.

Study design

The participants were kept on fast at least 10 h (over night). They were stopped taking water 1 h before administration of the drug. A single dose of 200 mg (in hard gelatin capsules) of the test product, salicylidine-sulfamethoxazole-

Zn(II) (sal-smz-Zn.H_2O) prepared according to the reported method [3], was administered to the participants along with 240 mL of water. The participants were kept fasting for 5 h after administration of the drug. They were allowed to take water 1 h after administration of the drug during this fast; after that standard meals were served throughout the study.

5 milliliter of venous blood samples were collected from each volunteer by using disposable syringes under aseptic conditions and transferred in labeled pre-heparinized vacutainer tubes. The volunteers were cooperative; no one dropped out. Blood samples were collected just before (blank) and after 0.16, 0.33, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 h of drug administration. The blood samples were centrifuged at 3000×g and 4 °C for 3 min. Plasma was separated by using micro pipette and stored at −20 °C in polystyrene crystal tubes sealed and wrapped with aluminum foil, separately, and labeled accordingly until analyzed.

HPLC method

All the chemicals used for HPLC analysis were of analytical reagent grade and were obtained from E. Merck, Germany. The test compound, sal-smz-Zn.H_2O, was prepared according to the reported method [4]. The HPLC system consisted of: a quarternary pump (G1311A), a UV-visible diode array detector (DAD G1315B), a column oven (TCC G1316), and an auto sampler (ALS G1319A) all from Agilent (1200 series), USA. The column used was Shim-Pack ODS 5 µm (4.6×250 mm). The mobile phase used was a methanol-acetic acid (100:05) mixture. The flow rate, detection wavelength, and injection volume used were 1 mL min^{-1}, 257 nm, and 5 µL, respectively. The new method has to be developed because none of the reported methods could separate salicylaldehyde and smz, the in vivo decomposition products of sal-smz-Zn.H_2O, simultaneously.

The standard stock solution of sal-smz-Zn.H_2O was prepared by dissolving 100 mg in 100 mL of the mobile phase and filtered. A volume of 20 mL from this solution was diluted to 100 mL with the mobile phase and 1 mL of the resulting solution was added to blank plasma (1 mL) to obtain a concentration of 0.1 mg mL^{-1} of the analyte. To the above solution, acetic acid (1 mL) was added. The contents were vortex-mixed for 90 s and centrifuged at 3000×g and 4 °C for 3 min. The supernatant was separated with the micro pipette and used for analysis as the standard. The HPLC was performed by use of sulfadimethoxine (Sigma-Aldrich) as an internal standard.

Specimen analysis

The test plasma sample (1 mL) was thawed quickly under cold water, then promptly but briefly vortex-mixed and proceeded as for the standard preparation. The newly validated HPLC method was used for the analysis of plasma samples and concentration of smz was determined. Typical chromatograms of blank and sample plasma are shown in **Fig. 1**.

Pharmacokinetic study

Concentration-time curves were plotted and following parameters were determined:

AUC_{0-t}, the area under the curve from time zero to time t; $AUC_{0-\infty}$, the area under the curve from time zero to time infinity

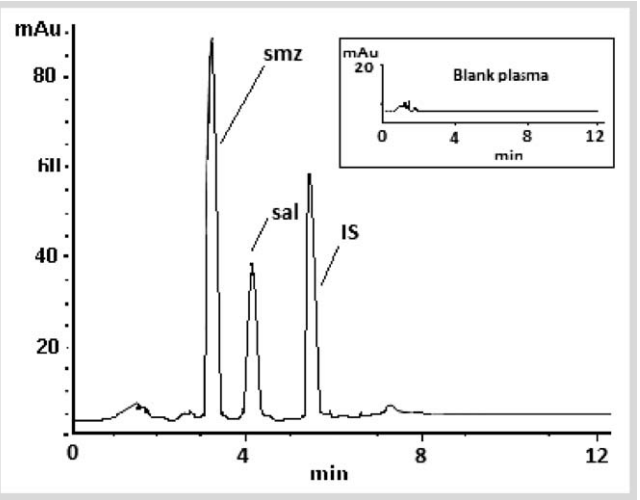

Fig. 1 Typical chromatograms of blank plasma and the plasma spiked with the test compound and internal standard (sulfadimethoxine).

Table 1 Validation parameters of sal-smz-Zn.H_2O in the plasma phase at 3 different concentration levels. The concentration units are µg mL^{-1}.

Conc level	Precision (CV)		Recovery (%)	LOD	LOQ
	Within day	Between days			
100	0.13	0.31	96.01	8.4	16.9
200	0.18	0.35	96.32		
500	0.12	0.23	96.41		
Resolution of closely eluting peaks:				1.98	
Symmetry factor for smz:				0.98	
Capacity factor for smz:				4.2	

using the formula $AUC_{0-\infty} = AUC_{last} + C_t/k_e$; $t_{½} = 0.693/k_e$, the half-life of the drug; C_{max}, the peak drug concentration; t_{max}, the time to peak drug concentration; the clearance $Cl = Dose/AUC_{0-\infty}$; and volume of distribution $V_d = Cl/k_e$. The area under the concentration-time curve was calculated by the linear trapezoidal method. The terminal rate constant, k_e, was determined by regression analysis of at least 3 data points in the terminal phase. The statistical analysis was performed by use of Statgraphics® 5.1.

Results and Discussion

HPLC method

Method validation was carried out in the plasma sample as per reported method [6]. The performance parameters thus obtained are given in **Table 1**. The stability study of drug in mobile phase as well as in plasma was carried out and it was found that the chromatogram did not change significantly over the period of 72 h, i. e., there was no significant change in the peak position as well as the peak areas in mobile phase and plasma.

The method was found to be specific for the determination of smz as the reproducibility of measurement (CV = 0.01) of 5 different plasma samples spiked with the standard was very high and there was no interfering peak in the region. This was validated by analyzing some of the plasma samples by a reported method [7], and a good correspondence ($t < t_{95,5}$) was found between the 2 methods. However, the reported method could not detect salicylaldehyde. Therefore, the present method was considered superior to that.

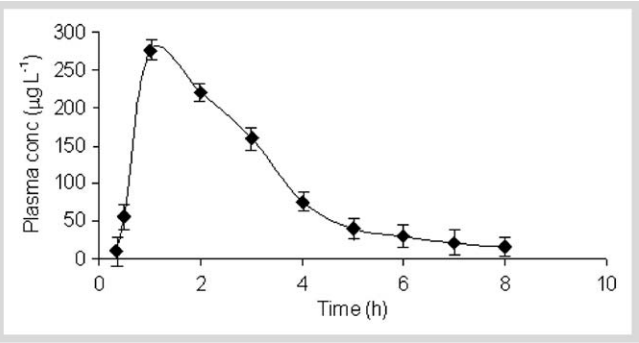

Fig. 2 Plasma concentration-time curve of free smz from sal-smz-Zn.H_2O (mean ± SD, n = 6).

The method was found to be linear ($R^2 > 0.999$) in the range 100–500 µg mL^{-1}. The lower limit of quantification (LOQ) in the plasma was found to be very low (16.919 µg mL^{-1}), with excellent precision (CV: 0.12–0.18 % within day; 0.23–0.35 % between days) and accuracy (96.01–96.41 % recovery). Other parameters also qualified the standard suitability criteria. Thus the method was considered to be suitable for the present study.

Safety of sal-smz-Zn.H_2O

The single dose of sal-smz-Zn.H_2O was well tolerated without any adverse effect (AE). Clinical assessment continued throughout the study, and no AE was reported.

Pharmacokinetic study

The validated HPLC method was applied for study of pharmacokientics The retention time for smz from sal-smz-Zn.H_2O in spiked plasma was 3.2 min. Similar peaks with similar retention time were found in the samples collected from the subjects of this study. Resolution between the closely eluting peaks was 1.98 min. Thus separation and determination of smz was carried out successfully by the use of this method.

The plasma concentration-time curve is shown in **Fig. 2**. The area under the curve ($AUC_{0-\infty h}$) was 779 h µg L^{-1} and C_{max} was found to be 280 µg L^{-1} at t_{max} 1.30 h for smz from the single 200 mg oral dose of sal-smz-Zn.H_2O which is about 0.70 % of the administered dose. In case of smz, normally, it is about 8 % after 1.45 h at 400 mg oral dose [7] which suggests that smz is released slowly with a rapid onset effect. The $t_{1/2}$ of smz from sal-smz-Zn.H_2O was 1.64 h as compared with 9–11 h that of the parent drug [8]. The V_d and Cl values for sal-smz-Zn.H_2O were 0.57 L kg^{-1} and 0.24 L h^{-1}, respectively to that of 0.25 L Kg^{-1} and 0.19 L h^{-1} [8] of the parent drug. The elimination of smz from sal-smz-Zn.H_2O followed the first order kinetics with $R^2 = 0.984$. The larger V_d and Cl values of sal-smz-Zn.H_2O, possibly due to its higher lipophilicity and tissue binding capability, as compared to that of the parent drug show that the complex can exhibit prolonged antimicrobial effect with rapid clearance. It can be concluded that sal-smz-Zn.H_2O acts as a prodrug with sustained release profile.

Conflict of Interest

There was no conflict of interest involved in this work.

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