



RESEARCH ARTICLE

**Influence of mercury chloride-renal failure on pharmacokinetics of sulphamethoxazole after oral administration in mice**

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**Özet**

**Yarsan E, Altıntaş A, Essiz D, Altıntaş L, Pekcan M, Ciftci G, İbrahim IG.** Farelerde Cıva Klorür ile Oluşturulan Böbrek Hasarının Oral Yolla Uygulanan Sülfametaksazolün Farmakokinetiği Üzerine Etkisi. *Eurasian J Vet Sci*, 2010, 26, 1, 21-24

**Amaç:** Bu çalışmada, deneysel olarak cıva klorür ile böbrek hasarı oluşturulmuş farelerde 100 mg/kg dozda oral yolla uygulanan sülfametoksazolün farmakokinetiği değerlendirildi.

**Gereç ve Yöntem:** Cıva klorür farelere 3 ve 6 mg/kg dozlarında verildi. Plazma sülfametaksazol konsantrasyonları spektrofotometre ile ölçüldü. Plazma konsantrasyon-zaman verileri dikkate alındığında ilacın 2 kompartmanlı açık modele uygun olduğu görüldü.

**Bulgular:** Böbrek hasarı ile ilişkili olarak sülfametoksazolün emilim (AUC and  $t_{1/2a}$ ) ve eliminasyon ( $t_{1/2\beta}$  and MRT) fazlarında önemli bulgular ( $p<0.05$ ) gözlemlendi. Hasara bağlı olarak eğrinin altında kalan alan değeri düşerken, ortalama kalış zamanında artış tespit edildi ( $p<0.05$ ).

**Öneri:** Böbrek hasarlı hastalarda, sülfametoksazolün doz ve uygulama aralıklarının hasarın derecesine göre belirlenmesi çok önemlidir.

**Abstract**

**Yarsan E, Altıntaş A, Essiz D, Altıntaş L, Pekcan M, Ciftci G, İbrahim IG.** Influence of mercury chloride-renal failure on pharmacokinetics of sulphamethoxazole after oral administration in mice. *Eurasian J Vet Sci*, 2010, 26, 1, 21-24

**Aim:** The effects of renal failure on the pharmacokinetics of sulphamethoxazole were investigated after oral administration of 100 mg/kg of the drug using a mice model of mercury chloride-induced renal failure.

**Materials and Methods:** Mercury chloride was given to mice at the doses of 3 and 6 mg/kg. Plasma sulphamethoxazole concentrations were measured by spectrophotometer. Plasma concentration-time data were fitted to a two-compartment open model.

**Results:** Significant findings ( $p<0.05$ ) were observed for absorption (AUC and  $t_{1/2a}$ ) and elimination ( $t_{1/2\beta}$  and MRT) phases of sulphamethoxazole related with renal failure. Also area under the curve value decreased, and mean residence time value increased with renal failure ( $p<0.05$ ).

**Conclusion:** Study result showed that dosage and administration intervals of sulphamethoxazole were important for patients with renal failure.

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## ► Introduction

Sulphamethoxazole is a member of the sulfonamide group. It is used worldwide in the treatment of bacterial and protozoa infections, particularly in combination with other drugs (especially trimethoprim) in treating acute urinary tract infections and malaria. Sulphamethoxazole usually is taken two or three times daily, with or without meals. It should be taken with 6 to 8 ounces of liquid to prevent crystals from in the urine (Foltzer and Reese 1987, Bevil 1988, Bywater 1991, Prescott and Baggot 1993, Smith and Keith 2000, Maddison and Watson 2002, Kayne and Jepson 2004).

Sulphamethoxazole and trimethoprim have very similar pharmacokinetic properties. The individual pharmacokinetic profile of one agent is not altered in the presence of another agent. They are both rapidly and almost completely absorbed from the gastro-intestinal tract following oral administration. Peak plasma levels are attained within 1-4 hours. Approximately 65% of the sulphamethoxazole is bound to plasma proteins and the plasma half-life is 6-12 hours. Following absorption, distribution and in some cases metabolic transformation, sulphamethoxazole is excreted in urine, feces, bile, milk, sweat and tears. However, the kidney is primarily involved in excretion of this drug. Both sulphamethoxazole and trimethoprim are almost exclusively eliminated by renal excretion via glomerular filtration and tubular secretion processes. In patients with severely impaired renal function dosage adjustment is required (Barnett and Bushby 1970, Mandell and Sande 1990, Cockerill and Edson 1991, Smilack 1999, Spoo and Riviere 2001, Maddison and Watson 2002, Bishop 2005).

Drugs are eliminated from the body by metabolism (mainly in the liver and/or excretion mainly via the kidney by glomerular filtration and/or renal tubular secretion). It has been reported that the total body, renal, and nonrenal clearances of drugs that were eliminated mainly by metabolism or mainly by renal excretion were altered in animals with renal failure. Therefore, it could be expected that the pharmacokinetics and pharmacodynamics of drugs usually altered in the renal failure (Bevil 1988, Kaneko et al 1997, Smith and Keith 2000, Altintas et al 2001, First 2003). Sulphanomides are commonly used antibiotics in veterinary medicine. There is no article concerning the pharmacokinetic profile of this drug in renal failure.

The purpose of this study was to investigate pharmacokinetics of orally administrated sulphamethoxazole in mice with renal failure induced by mercury chloride.

## ► Materials and Methods

### *Animals and study design*

One hundred and twenty Swiss albino mice (30-35

g) were used in the study. They were assigned into four study groups, each group consisting of 30 mice. Sulphamethoxazole was given at a dose of 100 mg/kg of body weight as intraperitoneal (Group I) and orally (Group II). Sulphamethoxazole with same doses were given orally to mice (Groups III and IV). Mercury chloride ( $\text{HgCl}_2$ ) was also given to these, groups III and IV previously as 3 mg/kg b.w. and 6 mg/kg b.w. intraperitoneal for inducing of renal failure.  $\text{HgCl}_2$  was given to animals in group III and IV to cause kidney damage by intraperitoneal route before 24 h of drug administration. Blood and urine samples were taken before and after 24 h of  $\text{HgCl}_2$  administration to determine the possible effect of drug on kidney. The Ethics Committee of the Faculty of Veterinary Medicine (University of Ankara, Ankara, Turkey, report no: 2005/23) approved the study protocol.

Blood samples were collected by cardiac puncture into sterile glass test tubes with anticoagulant at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 18 and 24 h after sulphamethoxazole administration. Five mice were used at each period. Plasma was obtained by centrifugation of collected blood samples (2500 rpm for 15 min at room temperature) within 2 hour of blood collection and stored at  $-20^\circ\text{C}$  for further analysis. Plasma concentrations of sulphamethoxazole were determined spectrophotometrically as described by Hammond (1977).

### *Pharmacokinetic analysis*

Plasma concentration time data were fitted to a 2-compartment open model with the first order absorption for kinetic analysis. Pharmacokinetic variables were calculated using a computer program (PK CALC) based on equations described by Shumaker (1986), and based on the equations described by Wagner (1975).  $C_{\text{max}}$  and  $t_{\text{max}}$  values were determined by direct observation of the data.

### *Biochemical analysis*

Plasma urea and creatinine levels were determined by an autoanalyser using commercial test kits. Urine samples were obtained directly from the bladder by sterile injectors. Because of decreased urine volume, samples were pooled and mixed with sample buffer and further denaturation of proteins was done by heating at  $95^\circ\text{C}$  for 5 minute. Proteins were separated by SDS-PAGE (10% acrylamide gel, tris-glycine buffer pH 8.3 and 20 mA) (Laemli 1970).

Appearance of the protein profiles after electrophoresis were shown using coomassie blue R-250, protein dye. Molecular weight of the urine proteins were determined using standard proteins markers.

### *Statistical analysis*

Data were analyzed statistically by one-way analysis of variance (ANOVA). When significant treatment effects were detected, DUNCAN's multiple range test was used to identify specific differences between treatment means (of probability level of 5.0%).

## ► Results

In this study, kidney damage was induced by HgCl<sub>2</sub>, given in two different doses (3 mg/kg and 6 mg/kg body weight). Effects of HgCl<sub>2</sub> on kidneys were confirmed by plasma urea and creatinine level (Table 1). Creatinine and BUN levels in Groups III and IV were higher than control values (p<0.05). Also, kidney damage in animals was proved via the calculation of the plasma urea nitrogen and creatinine levels, and the electrophoretic investigation of the urine proteins (Figure 1 and 2). Densitometric analysis of protein patterns were also different related to dose.

Protein existence in the urine was also detected in a few mice in control group (Figure 1). Proteinuria was accepted as a sign of renal tubular or tubular interstitial damage (see the bands with molecular weight of strong bands 36 and 29.5 kDa; and as weak bands 66.0; 62.5 and 25.5 kDa). In addition, proteinuria was observed in animals administered HgCl<sub>2</sub> with the dose of 3 mg/kg.

Sulphamethoxazole was given at the dose of 100 mg/kg and plasma sulphamethoxazole levels were measured. AUC<sub>(0-24)</sub>, t<sub>1/2α</sub>, t<sub>1/2β</sub>, t<sub>1/2α'</sub>, MRT, C<sub>max</sub>, t<sub>max</sub> and F values were evaluated as pharmacokinetic parameters (Table 2). In all study groups, t<sub>max</sub> was determined as

30 minutes. High dose of HgCl<sub>2</sub>, caused a significant decrease (p<0.05) on C<sub>max</sub>. Nevertheless, t<sub>1/2α</sub>, t<sub>1/2β</sub> and MRT were increased upon exposure to HgCl<sub>2</sub> with the elevated doses and kidney damage. Changes of AUC<sub>(0-24)</sub>, t<sub>1/2α</sub>, t<sub>1/2β</sub>, MRT and C<sub>max</sub> values were statistically significant (p<0.05, Table 2).

## ► Discussion

The serum levels and pharmacokinetic results obtained in mice with varying degrees of renal failure in the present study are predictable from the known pharmacokinetic parameters of sulphamethoxazole.

Varying degrees of kidney failure was induced by administering different doses of mercury chloride in Group III and IV. This evaluation was based on determination of plasma creatinine and BUN levels (Table 1). These results indicate that there is correlation between administered dose of the HgCl<sub>2</sub> and the severity of kidney failure. Presence of proteinuria and electrophoretic profile of proteinuria supported these findings (Figure 1 and 2). Calculation of the molecular weight of the proteins and the finding that the common proteins with low molecular weight indicate that the disease originates from tubular region. Presence of low molecular weight proteins in the urine is a symptom of tubular disease (Bazzi et al 1997, Kaneko

Table 1. Creatinin and BUN levels in control and experiment Groups III and IV with mercury chloride-induced renal failure.

Parameters	Group I	Group III (3 mg/kg HgCl <sub>2</sub> )	Group IV (6 mg/kg HgCl <sub>2</sub> )
Creatinin mg/dL	0.75	1.30	1.68
BUN mg/dL	17.2	50.5	67.8

Table 2. Pharmacokinetic parameters of sulphamethoxazole after administration of 100 mg/kg to all study groups.

Parameters	Group I	Group II	Group III	Group IV
AUC <sub>0-24</sub> µg.h/mL	864±69.3 <sup>a</sup>	694±55.8 <sup>b</sup>	775±35.0 <sup>ab</sup>	498±65.1 <sup>c</sup>
t <sub>1/2α</sub> h	1.44±0.09 <sup>a</sup>	1.38±0.16 <sup>a</sup>	1.97±0.38 <sup>b</sup>	2.47±0.41 <sup>c</sup>
t <sub>1/2β</sub> h	6.63±1.96 <sup>a</sup>	22.2±6.94 <sup>b</sup>	5.02±0.40 <sup>a</sup>	19.9±10.9 <sup>b</sup>
t <sub>1/2α'</sub> h	0.13±0.05	0.43±0.09	0.14±0.01	0.43±0.66
MRT h	5.26±0.68 <sup>a</sup>	17.2±9.53 <sup>ab</sup>	9.59±3.56 <sup>a</sup>	25.7±12.1 <sup>b</sup>
C <sub>max</sub> µg/mL	268±33.8 <sup>a</sup>	252±5.97 <sup>a</sup>	153±12.9 <sup>b</sup>	69.0±8.48 <sup>c</sup>
t <sub>max</sub> min	30	30	30	30
F %	-	80	89	57

a, b, c; same rows with different letters are statistically significant (p<0.05). t<sub>1/2α</sub>: absorption half life, t<sub>1/2α'</sub>: distribution half life, t<sub>1/2β</sub>: elimination half life, MRT; mean residence time, AUC<sub>0-24</sub>: area under the concentrations time curves, t<sub>max</sub>: time to maximum concentration, C<sub>max</sub>: maximum concentration.

et al 1997, First 2003). Severity of renal failure is in parallel with the given dose of HgCl<sub>2</sub> and plasma urea and creatinine levels (Table 1) besides urine protein electrophoresis confirms the degree of kidney failure (Figures 1 and 2).

A linear, two compartment open model characterized by first-order elimination best describes the disposition of sulphamethoxazole. After administration of the drug 100 mg/kg body weight t<sub>max</sub> was found to be 30

minutes in all groups. C<sub>max</sub> was also significantly low in Group III and IV. In parallel with C<sub>max</sub>, AUC was decreased (P<0.05, Table 1). Similar findings were observed in other studies conducted with drugs other than sulphanomides. In a study, (Bae et al 2006) about the kinetics of oxazolidinone found that AUC and C<sub>max</sub> levels were decreased because of renal failure. Similarly, Lee et al (2000) evaluated the pharmacokinetics of cyclosporine and they found a decrease in C<sub>max</sub> because of kidney failure.

In our study,  $t_{1/2a}$  and  $t_{1/2\beta}$  levels in group IV were higher ( $p < 0.05$ ) than control value. Sulphamethoxazole was absorbed and excreted slowly. Drug absorption and excretion was delayed consequence of renal failure. Similar findings were found by the studies of Falcoz et al (1987) and Welling et al (1975) in kidney failure. In correlation with the delay of excretion, MRT levels were raised. Especially in the Group IV, which has the highest severity of kidney failure, MRT levels were 25.70.

### ► Conclusions

Kidneys are one of the organs that have effects on the behavior of the drugs. Changes in the kidney failure

have direct effects on drug pharmacokinetics. In this study experimental kidney failure was made by administration of mercury chloride. Kidney failure was confirmed by determining plasma creatinine, BUN and urine protein electrophoresis. Significant pharmacokinetic alterations were observed in drug absorption, distribution and excretion. Main alterations were seen in the parameters related to absorption and distribution. Because of the above factors to be kept in mind, when planning dosage and administration intervals of the sulphamethoxazole of patients with renal failure.

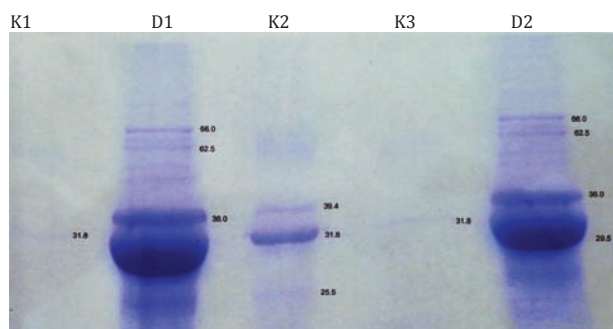


Figure 1. Urine protein bands (K1, K2, K3) from Group I, Group II and Group III (D1, D2) (3 mg/kg HgCl<sub>2</sub>).

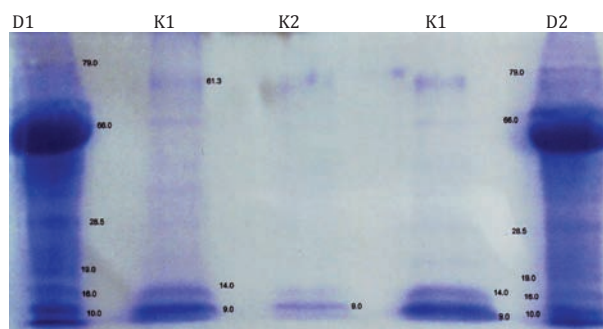


Figure 2. Urine protein bands (K1, K2) from Group I, Group II and Group IV (D1, D2) (6 mg/kg HgCl<sub>2</sub>).

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