한국임상수의학회지 14(1) : 42-47 (1997)
Korean J. Vet Clin Med. 14(1) : 42-47 (1997)

Pharmacokinetics of Sulfadiazine/Trimethoprim from Bronchial Secretion in Pigs.

Kil-soo Kim[1], Hyo-in Yun*
Dept. of Laboratory Animal Research, ASAN Institute for Life Sciences
*College of Veterinary Medicine, Chungnam National University**

돼지의 기관분비에서 Sulfadiazine과 Trimethoprim의 약물동태학

김길수[1] · 윤효인*
아산생명과학연구소 실험동물연구실
충남대학교 수의과대학*

요 약 : 돼지의 호흡기감염은 양돈산업에서 과밀한 사육으로 인하여 발생되는 주요한 질환중의 하나이다. 호흡기감염을 일으키는 병원체들은 주로 기관분비물과 점액에 상주하는 것으로 알려져 있다. 그러므로 호흡기감염의 치료를 위하여 기관분비물내에 항균약제의 농도는 적절하게 유지되어져야 한다. sulfadiazine (SDZ)과 trimethoprim (TMP)의 혼합제제는 전세계적으로 돼지의 호흡기감염의 치료에 널리 사용되어지고 있으나, 기관분비물에서 약물의 동태학적 특성에 관한 연구는 거의 찾아 볼 수가 없었다. 본 연구는 돼지의 좌측전대정맥으로 SDZ/TMP혼합제제를 일회 투여한 후 형성되는 약물동태학적 매개변수를 알아보기 위하여 실시되었다. 혈장과 기관분비물에서 SDZ/TMP농도는 HPLC (High Performance Liquid Chromatography)로 측정하여 약물동태학적 매개변수를 산출하였다. 혈장에서 SDZ와 TMP의 약물동태학적 매개변수는 2-compartment와 1-compartment모델에 적합하였다. 혈장내 TMP의 반감기(0.90±0.06시간)가 SDZ(7.25±1.09시간)에 비하여 매우 짧은 점이 주요한 차이점으로 볼 수 있었으며, 더욱이 투여후 8시간이후에서는 측정이 되지 않았다. 기관분비물에서 SDZ의 농도는 전체 실험기간(0.5~32시간)동안 거의 일정하게 유지되었고, 16시간후에서는 혈장농도보다 높은 수준을 나타내었다. TMP농도는 투여후 2~3시간에서 혈장농도보다 높게 유지되었다. 이러한 결과를 토대로 호흡기감염의 처치와 예방에 있어서 약물의 용량 및 투여빈도를 결정하는데 응용되어 질 수 있을 것이다.

Key words : pharmacokinetics, pig, sulfadiazine, trimethoprim

Introduction

Potentiated sulfonamides, which are combined with a dihydrofolate reductase inhibitor such as trimethoprim (TMP), provide effective antimicrobial therapy[7]. Sulfonamide (5parts)+trimethoprim (1part) mixtures have been used in the treatment and prophylaxis of Pneumocystis carinii pneumonia, Shigella sp enteritis, systemic Salmonella infections, and many others[5,6,12,14,18,22].

Sulfadiazine (SDZ, 5parts)+trimethoprim (TMP, 1part) mixture is effective against many gram-positive and gram-negative bacteria, including *Escherichia Coli, Streptococci, Salmonella, Proteus, Bordetella, Staphylococci* and *Nocardia*[9]. Important swine diseases treated with sulfonamides or sulfonamide/trimethoprim combinations have been colibacillosis, atrophic rhinitis and (enzootic) pneumonia[3].

In pigs, the most common pathogens of pneumonia and bronchopneumonia include *Bordetella bronchiseptica, Haemophilus pleuropneumoniae, Pasteur-*

[1]Corresponding author.

ella spp, and *Streptococci*[8,13]. The minimal inhibitory concentration (MIC) *in vitro* of SDZ and TMP against *bordetella. bronchiseptica, pasteurella. multocida, salmonella. suis* and *haemophilus. pleuropneumoniae* ranged from 2 to 10 μg/ml, from 0.06 to 2 μg/ml, respectively[10,15,16,19].

On the other hand, the proper antibacterial therapy requires appropriate concentrations of antibacterial agents at the site of infection. Generally, in acute pneumonia or acute exacerbations of chronic bronchitis, the site of infection is in the bronchial epithelial areas and pathogens are mainly located in the bronchial secretions and mucosa[2,11,17,20]; consequently, concentration of antibacterial agents in bronchial secretions would be more important in assessments the efficacy of antibacterial agents. Nevertheless, little is known about the concentrations of SDZ and TMP in bronchial secretions in pigs.

Thus, the purpose of the study reported here was to determine whether therapeutic concentrations of SDZ and TMP could be achieved in bronchial secretion in pigs.

Materials and Methods

Animals and surgical preparations

The investigations were carried out in 10 pigs, commercially raised 3 months old, which obtained from local farms. The procedures of surgical preparations were as follows; a pig was sedated with 250 mg of Nembutal i.v. injection through the left anterior vena cava, and anesthesized with Halothane inhalation for chronic tracheostomy. The cuffed tracheostomy tube (Portex, France, ID: 10.0 mm, OD: 13.7 mm) was inserted into the trachea through the neck, with insertion of an indwelling tube to facilitate passage of evacuation of bronchial secretions. All experiments were performed 24 hours after tracheostomy surgery.

Drugs

A combination of the sulfadiazine/trimethoprim (Tribrissen, Burroughs Wellcome Co, Research Triangle Park, NC) was obtained as a 24% (200 mg of sulfadiazine and 40 mg of trimethoprim in 1 ml) sterile suspension for i.v. injection. The preparation was administered via the left anterior vena cava at a dose of 30 mg of combination/kg of body weight as bolus.

Blood sampling

Blood samples, approximately 2 ml each, were taken from the right anterior vena cava under light halothane anesthesia 0, 0.5, 1, 2, 3, 4, 8, 16, 24 and 32 hours after SDZ/TMP intravenous administration. The blood samples were treated with EDTA as anticoagulant and centrifuged at 2,000×g for 10 mins to separate plasma. 0.2 ml aliquots of plasma were stored at -20°C until assay.

Bronchial secretions sampling

Bronchial secretions samples were collected in a sanitary tampon every time blood was taken. Volumes of bronchial secretions were calculated from differences between pre- and postsampling weights of sanitary tampons, and 2 ml of distilled water added to tampon soaked up the bronchial secretion and extracted the secretions in sonicator (UN-85005, Shibuya Electric Mfg Co, Japan). 0.2 ml aliquotes of distilled water-diluted bronchial secretions were stored at -20°C until assay.

SDZ and TMP determination

The concentrations of SDZ and TMP in plasma and bronchial secretions were determined concomitantly by use of high-performance liquid chromatography (HPLC) equipped with UV detector (270 nm)[4]. The HPLC system consited of a pump (LC-6A, Shimadzu Co, Kyoto, Japan), analytical column (Lichrospher RP-8, 5 μm, 250×4 mm ID, Merck, Darmstat, Germany), UV-detector (SPD-6A, Shimadzu Co). Plasma and bronchial secretion fluid protein were removed by mixing with equal volume of 0.5 N perchloric acid followed by centrifugation at 5,000×g for 2 mins. The supernatant was filtered through 0.45 m filter and injected into the HPLC system (100 μl). The mobile phase consisted of a mixture of 0.05 M phosphate buffer and acetonitril (85/15, v/v). The flow rate was 0.8 ml/min. Under these conditions, the retention times of SDZ and TMP were 23 mins

and 18 mins, respectively.

Pharmacokinetic analysis

Plasma concentration of SDZ, after IV injection of SDZ was best described by a two-compartment model, whereas that of TMP was fit by a one-compartment model.

Pharmacokinetic parameters

AUC(μg/h/ml) – Area under the concentration-time curve from zero to infinity

$T_{1/2}(\alpha)$ (h) – Absorption half-life

$T_{1/2}(\beta)$ (h) – Elimination half-life

V_d SS(l/kg) – Volume of the body fluids having the same concentration as that of blood, based on average steady state of plasma level

C_{max} (μg/ml) – Maximum plasma concentration

Cl(ml/h/kg) – Clearance

A(μg/ml) – Zero-time plasma drug concentration, intercept of regression line of distribution phase

B(μg/ml) – Zero-time plasma drug concentration, intercept of regression line of elimination phase

$\alpha(h^{-1})$ – Overall distribution rate constant

$\beta(h^{-1})$ – Overall elimination rate constant

Statistical analysis

The unpaired student's t-test was used for the detection of significant differences between the pharmacokinetic parameters of plasma and bronchial secretory SDZ or TMP; $p<0.05$ was considered significant.

Results

The mean plasma and bronchial secretion concentration-time curves of SDZ and TMP in pigs are presented in Fig 1 and Fig 2, respectively. Concentrations of SDZ and TMP in plasma were highly maintained than bronchial secretion. Concentration in plasma gradually decresed in process of time, whereas, concentration in bronchial secretion constantly maintained until 32 hours after injection. Levels of plasma concentration of SDZ and TMP were reversed in 16 and 1.5 hours with bronchial secretion, respectively.

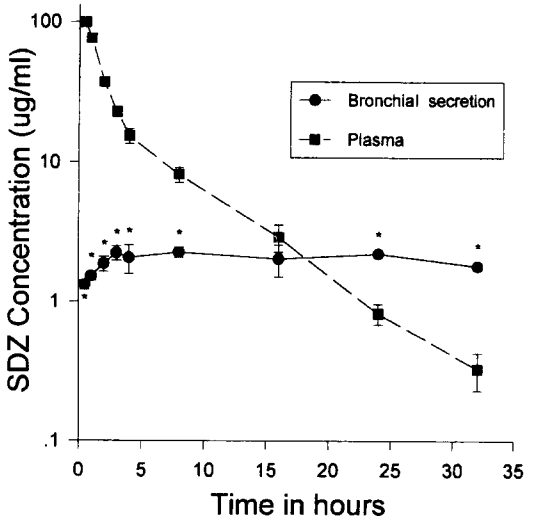

Fig 1. Plasma and bronchial secretions drug concentration vs time in pigs given a single I.V of 25 mg/kg of sulfadiazine (n=4).

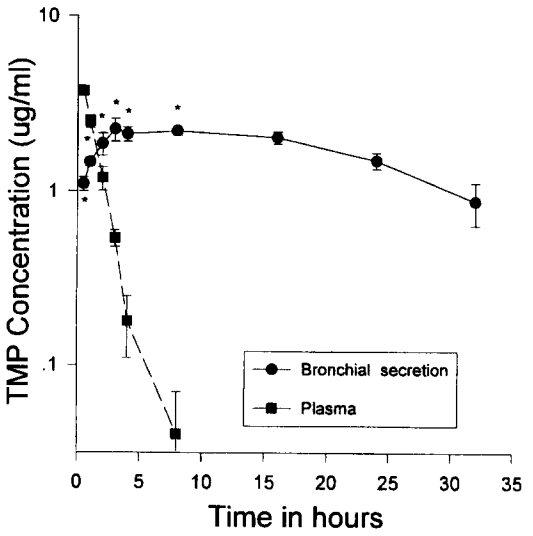

Fig 2. Plasma and bronchial secretions drug concentrations vs time in pigs given a single. I.V of 5 mg/kg of trimethoprim (n=4).

The pharmacokinetic parameters of SDZ and TMP are shown in Table 1 and Table 2, respectively. $T_{1/2}$ (β; Elimination half-life) of SDZ was apparently longer than that of TMP. Moreover, V_dSS (l/kg; Volume

Table 1. Pharmacokinetic profiles and hybrid constants for 2- compartmental model of sulfadiazine (25 mg/kg) in four pigs after I.V. injection

Profiles	1	2	3	4	mean	S.E.M.
AUC (μg/h/ml)	308.95	351.36	260.72	341.55	315.65	20.43
$T_{1/2}$ (α) (h)	0.93	0.86	0.73	1.14	0.92	0.09
$T_{1/2}$ (β) (h)	6.48	7.75	5.16	9.61	7.25	0.95
V^{ss}_d (l/kg)	0.180	0.156	0.156	0.212	0.176	0.013
Cmax (μg/ml)	138.95	160.57	160.18	118.11	144.45	10.73
Cl (ml/h/kg)	0.081	0.071	0.096	0.073	0.08	0.01
A (μg/ml)	123.58	145.26	145.85	105.99	130.71	9.58
B (μg/ml)	15.37	15.32	14.33	12.12	14.29	0.76
$\alpha(h^{-1})$	0.75	0.81	0.95	0.61	0.78	0.07
$\beta(h^{-1})$	0.11	0.09	0.13	0.07	0.10	0.01

Table 2. Pharmacokinetic profiles for 1-compartmental model of trimethoprim (5 mg/kg) in four pigs after I.V. injection.

Profiles	1	2	3	4	Mean	S.E.M.
AUC (μg/h/ml)	6093	7.79	5.96	7.64	7.08	0.42
K_{10} (h^{-1})	0.76	0.68	0.79	0.90	0.78	0.05
V_dSS(I/kg)	0.947	0.949	1.059	0.727	0.921	0.070
Cmax (μg/ml)	5.28	5.27	4.72	6.88	5.54	0.47
Cl (ml/h/kg)	0.72	0.64	0.84	0.65	0.71	0.05
$T_{1/2}$ (h)	0.91	1.02	0.88	0.77	0.90	0.05

of the body fluids having the same concentration as that of blood, based on average steady state of plasma level) and Cl (ml/h/kg; Clearance) values of SDZ were significantly lower than that of TMP.

Discussion

Bronchial secretions concentrations of SDZ and TMP increase more slowly than in plasma, achieving lower peaks and exhibiting longer half-lives. The TMP and SDZ may persist in bronchial secretions while they are cleared from plasma and local bronchial secretions levels might exceeded plasma concentration.

The results showed a poor penetration of the SDZ in contrast with the TMP, which quickly reached concentrations higher than the corresponding plasma level. The difference in penetration of TMP and of SDZ is attributable to the highly protein-bound SDZ.

Antibacterial agents kinetics in bronchial secretions include penetration, accumulation, and elimination of the SDZ and TMP[17]. Among drug factors influencing the antibacterial agents penetration into the respiratory tract, the role of protein binding, lipid solubility, and degree of ionization have been well documented[1,17,21]. Because TMP is more lipid-soluble than SDZ, it has a larger volume of distribution than the latter drug.

Intravenous administration of Tribrissen 30 mg/kg resulted in concentrations of TMP >2 μg/ml for more than 24 hours in bronchial secretions and which may be recommended for treatment and prophylaxis of respiratory tract infections caused by susceptible organisms (MIC< 2 μg/ml).

A significant correlations between effective antibacterial activity in the bronchial secretions and the efficacy of TMP and SDZ treatment have been predicted. In addition, satisfactory clinical responses correlated with bronchial levels of TMP inexcess of the MIC of the isolated pathogens.

The measurement of the concentrations of SDZ and TMP in bronchial secretions provide information on the transfer of antibacterial agents across the blood-bronchialveolar barrier. The experimental study

about pharmacokinetics of SDZ/TMP in the respiratory tract can be applicated in design of drug dosage in the treatment and prophylaxis of respiratory infection.

Conclusions

Respiratory infections are of prime importance in veterinary practice due to overcrowding raising environments in the swine industry. Pathogens causing respiratory infections are reported to be mainly located in the bronchial secretions and mucosa. Therefore, optimum concentrations of antibacterial agents in bronchial secretions have to be maintained during antibacterial therapy. Combination formulation of sulfadiazine (SDZ) and trimethoprim (TMP) have been extensively used in the swine respiratory infections worldwidely. But no study was carried out for the kinetic characteristics of the drugs in studies the pharmacokinetic profiles of SDZ/TMP in plasma and bronchial scretions after administering via the left anterior vena cava. TMP and SDZ concentrations in plasma and bronchial secretions were measured by HPLC and pharmacokinetic parameters were calculated with a computer program. The results indicated that pharmacokinetic profiles of SDZ in plasma were best described by a two-compartment model whereas that of TMP a one-compartment model. This dofferent pattern could be explainable by short half-life of TMP (0.90 ± 0.06 h) as compared to SDZ ($7.25\pm$ 1.09 h), together with unmeasurable TMP 4 to 8 hours after administration. Concentrations of SDZ in bronchial secretions were maintained throughout sampling period (0.5~32 hrs), with higher concentrations at 16 hr or later as to in plasma. In case of concentrations of TMP, measurable concentrtions at as early as from 2 to 3 hours after administration, in comparision with plasma. Signifiance of SDZ/TMP in bronchial secretions was discussed.

References

1. Baggot JD: Principles of pharmacokinetics. In: Principles of drug disposition in domestic animals. Baggot JD(ed). W.B. Saunders company. Philadelphia. 1977; 144-187.
2. Bergogne-Berezin E: Pharmacokinetics of antibiotics in respiratory secretions. In: Respiratory Infections: Diagnosis and Management. (2nd ed). pennington JE(ed). Raven Press. New York. 1988; 608-630.
3. Bevill RF: Sulfonamides. In: Veterinary pharmacology and therapeutics (6th ed). Booth LE and McDonald LE (eds). Iowa State University Press. Ames. Iowa. 1988; 785-795.
4. Bonazzi D, Andrisano V, Di Pietra AM, Cavrini V: Analysis of Trimethoprim-sulfonamide drug combinations in dosage forms by UV spectroscopy and liquid chromatography (HPLC). *Farmaco.* 1994; 49(6): 381-386.
5. Brander GC, Pugh DM, Bywater RJ, and Jenkins WL: Veterinary Applied Pharmacology & Therapeutics. 5th ed. Bailliere Tindall. London. W.B. Saunders. 1991; 489-496.
6. Bushby SRM: Sulphoamide and trimethoprim combinations. *J Am Vet Med Assoc.* 1980; 176: 1049-1053.
7. Bushby SRM and Hitching GH: Trimethoprim, a sulphonamide potentiator. *Br J Pharmacol Chemother.* 1968; 33: 72-90.
8. Clarke CR, Short CR, Corstvet RE, Nobles D: Interaction between *Pasteurella haemolytica,* sulfadiazine/trimethoprim, and bovine viral diarrhea virus. *Am J Vet Res.* 1989; 50(9): 1557-1567.
9. Dagorn M, Moulin G, Laurentie M, Delmas JM: Plasma and lung pharmacokinetics of trimethoprim and sulphadiazine combinations administered to broilers. *Acta Veterinaria Scandinavica.* 1991; 87: 273-275.
10. Goren E, De Jong WA, Doornebal P: Some pharmacokinetic aspects of four sulphonamides and trimethoprim and their therapeutic efficacy in experimental *escherichia coli* infection in poultry. *Vet Q.* 1984; 6: 134-137.
11. Hughes DTD: The use of combinations of trimethoprim and sulphonamides in the treatment of chest infections. *J Antimicrob Chemother.* 1983; 12: 423-434.
12. Hughes WT, McNabb PC, Markers TD, Feldman J : Efficacy of trimethoprim and sulfamethoxazole in the prevention and treatment of *Pneumocystis carinii. Antimicrob Agents Chemother.* 1974; 5: 289-293.
13. Koritz GD, Bevill RF, Bourne WA, Dittert LW : Disposition of sulfonamides in food-producing animals. Pharmacokinetics of sulfathiazole in swine. *Am J Vet Res.* 1978; 39: 481-486.

14. Mandell GL and Sande MA: Antimicrobial agents. In: The pharmacological basis of therapeutics (7th ed). Gilman AG, Goodman LS and Gilman A(eds). MacMillan Publishing Co. New York. 1985; 1066-1114.
15. Mengelers MJB, van Klingeren B, and van Miert ASJPA: *In vitro* susceptibility of some porcine respiratory tract pathogens to aditoprim, trimethoprim, sulfadimethoxine, sulfamethoxazole, and combinations of these gents. *Am J Vet Res.* 1990; 51: 1860-1864.
16. Nielsen P, Gyrd-Hansen N: Oral bioavailability of sulphadiazine and trethoprim in fed and fasted pigs. *Research in Vet Sci.* 1994; 56(1): 48-52.
17. Pennington JE: Penetration of antibiotics into respiratory secretions. *Rev Infect Dis.* 1981; 3: 67-73.
18. Richards RM, Xing JZ, Gregory DW, Marshall D: Mechanism of sulphadiazine enhancement of trimethoprim activity against sulphadiazine-resistant *Enterococcus faecalis. J of Antimicrobial Chemotherapy.* 1995; 36(4): 607-618.
19. Romvary A, and Horvay MS: Data on the pharmacokinetics of sulfonamide-trimethoprim combinations in suckling pigs. *Zbi Vet Med A.* 1976; 23: 781-792.
20. Shoaf SE, Schwark WS, Guard CL: Pharmacokinetics of sulfadiazine /trimethoprim in neonatal male calves: Effect of age and penetration into cerebrospinal fluid. *Am J Vet Res.* 1989; 50(3): 396-403.
21. Sigel CW: Pharmacokinetics of trimethoprim and sulfadiazine: absorption, distribution, metabolism, and excretion, in Proceedings. Symposium on trimethoprim/sulfadiazine. 1978; 16-23.
22. Walzer PD, Kim CK, Foy JM, Linke MJ, Cushion MT: Inhibitors of folic acid synthesis in the treatment of experimental *Pneumocystis carinii* pneumonia. *Antimicrobial Agents & Chemotherapy.* 1988; 32(1): 96-103.