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Effect of gender on the pharmacokinetics and metabolite formation of sulfamethazine in the rabbit

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토끼의 성차가 sulfamethazine의 약동학 및 대사산물 생성에 미치는 영향

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초록 : Sulfamethazine(SMZ)은 수의임상에서 감염증 치료 및 예방목적으로 많이 사용되고 있을 뿐 아니라 가축의 생산성 향상을 위해 남용되고 있는 주요한 항균제의 하나이다. SMZ의 생체내 대사 및 약물동태학적 특성은 동물의 종차에 따라 상이함이 잘 알려져 있으나 주요 실험동물 및 경제동물인 토끼에서 조사된 바는 매우 드물다. 한편 성차에 따른 약물대사의 차이는 rat를 비롯한 여러 동물에서 인정되고 있는데 대체로 숫컷이 암컷에 비해 대사능이 활발한 것으로 알려져 있다. 그러나 산양에서의 SMZ의 대사는 오히려 암컷이 더 활발하다는 보고도 있어, 여러 동물종에서 일률적으로 성차에 따른 약물대사를 설명할 수가 없다. 초식성의 습성을 가지고 있는 토끼에 있어 성차에 따른 SMZ의 대사 및 약물동태학적 특성이 다른 초식성 동물인 산양의 경우와 동일한 경향을 보이는지는 매우 흥미 있다할 것이다. New Zealand White 토끼에 SMZ을 이정맥에 35㎎/㎏를 주사한 후 미리 정해진 시간에 수거된 혈장 및 뇨(24시간)를 HPLC를 이용하여 분석하여 아래와 같은 약물동태학 및 대사적 특성을 얻었다.

1. 토끼에서의 SMZ의 주요 대사경로는 아세틸화(N_4AcSMZ)이었다. 두개의 수산화 대사산물(5OHSMZ, 6CH_2OHSMZ)도 생성되어 수산화 경로가 있음을 확인하였으나 양적인 관심에서 주요하지 않았다.
2. 토끼에서의 SMZ의 각 대사산물의 생성은 암수간의 성차에 따른 차이가 인정되지 않았다.
3. SMZ을 토끼의 이정맥에 투여(35㎎/㎏)하였을 때의 약물동태학적 특성은 1구1차 지수형 배설형태로 설명이 가능하였으며 암수에 따른 성차가 인정되지 않았다.
4. SMZ는 신속하게 N_4AcSMZ로 대사되었으며, N_4AcSMZ의 체외배설은 SMZ에 비해 매우 느렸으며 성차에 따른 배설속도의 차이를 인정할 수 없었다.

Key words : Sulfamethazine, Metabolites, Pharmacokinetics, HPLC, Sex differences, Species differences

Introduction

Sulfamethazine(SMZ) is one of the most widely used sulfonamides in veterinary medicine. It has been used for prophylactic purposes, treatment of various infections and growth promotion in a wide range of animal species. It is well known that significant differences in the metabolism of SMZ exist in both metabolic pathways and metabolic capacity among different animal species.[1] SMZ is known to be metabolized to hydroxy- and N_4-acetyl

metabolites, to a variable extent, depending on the species. For example, in cattle, goats and chicken, SMZ is more metabolized to 6-hydroxymethylsulfamethazine[2~4] from comparative viewpoints. In horses, 5-hydroxysulfamethazine is the major metabolite.[5] On the other hand, the acetylation pathway predominates in animal species such as man and the pig.[6,7] Among the various metabolites of SMZ, the hydroxylation metabolites were found to be microbiologically active, whereas the acetylated compounds were evaluated as inactive.[8] It is, therefore, important to have an insight in the metabolic pattern and pharmacokinetic profile of SMZ, not only for establishing appropriate dosage regimens but also in relation to the drug residues in edible tissues.

Sex-related differences in heapatic metabolism of various drugs have been reported in various animal species, but most particulary in the rat.[9] It was observed that, in general, the male rat has a higher rate of drug metabolism than the female. For SMZ, a remarkable influence of gender on its metabolism was reported in the goat. The direction of this sex differences was found to be opposed to the rat, the clearance of SMZ being much higher in female than in male goats.[10~12] For the rat, the sex-difference in the acetylation of SMZ was already reported.[13] Recently, we have also found in our preparative studies that there are remarkable sex-differences in the hydroxy metabolites formation of SMZ, the male being much higher in the metabolism than the female. SMZ is used rather extensively as a prophylactic and therapeutic agent in rabbits.[14] Detailed studies on the pharmacokinetics and its metabolic profiles of SMZ in rabbits are vary scarce. The aim of this study is to investigate the metabolic and pharmacokinetic profiles of SMZ in rabbits, with special interest in possible sex differences.

Materials and Methods

Animals : The experments were performed on clinically healthy six female(3.31 ± 0.24kg) and six male(3.40 ± 0.28kg) New Zealand White rabbits. The animals were kept indoors and fed with a diet of pelleted feed. Water was available *ad libitum*

Drugs and chemicals : A 3.5% sulfamethazine(AUU, the Netherlands) solution in water for injection was prepared by the Pharmacy of the Veterinary Faculty in the State University of Utrecht, The Netherlands. N_4-acetylsulfamethazine(N_4AcSMZ) was prepared by acetylation of SMZ as described by Vree and Hekster.[15] 5-Hydroxysulfamethazine(5OHSMZ) and 6-carboxymethylsulfamethazine(6CH_2OHSMZ) were synthetized or isolated at the Department of Vet. Pharmacol. and Toxicol. in the State Univ. of Utrecht, the Netherlands. Limpet acetone powder(glucuronidase/sulphatase, LAF, type Ⅰ : Platella vulgata) was obtained from Sigma(USA). The methanol used for HPLC analysis was from Rathburn(UK) and of HPLC quality. All other reagents were of analytical grade and obtained commercially.

Experimental procedures : Pharamcokinetic studies and the urinary excretion of the metabolites were studied on different occasions, using the same animals. SMZ was given as a bolus injection into the marginal ear vein of each animal at the dose level of 35mg/kg body wt. Heparinized blood samples were taken from the opposite marginal ear vein at predetermined intervals. The samples were centrifuged(4000rpm for 10min) and the plasma was stored at −20℃ until analysis. For the urine excretion study, the same amount of SMZ was given to the same route. Twenty four hr-urine was collected in metabolic cages. The urine collection vessels contained 2 ~3grams of mixture of boric acid and pyrosulfite.

Sample preparation :

Plasma : To 75 μℓ of plasma was added to 10 μℓ of internal standard(sulfapyridine, 100ppm). This mixture was extracted with 1.25mℓ ethyl acetate. The organic fraction was evaporated under a stream of N_2 gas. The residue was dissolved with 100 μℓ of mobile phase(10%-90%, MeOH-phosphate buffer; pH 6.67) before injection into the HPLC system.

Urine : To 100 μℓ of urine were added 20mg LAP and 1mℓ of acetate buffer(pH 4.5). After incubation of this mixture at 37℃ for 3 hrs, 60 μℓ of 4N NaOH, 500 μℓ of 0.5M phosphate buffer(pH 6.0), 200 μℓ of internal standard(sulfadimethoxine 100ppm) and 0.5 g of ammonium sulfate were added. The mixture was extracted and the organic fraction was evaporated under a stream of N_2 gas. The residue was redissolved with 500 μℓ of mobile phase for injection into HPLC.

HPLC analysis : The HPLC system consisted of a Spark Promis autosampler, a Kratos SP400 HPLC pump and a Kratos 783A variable UV detector. The stationary phase was a C_{18} column(10cm, particle size 5 μm, Chromspher, Chrompack, the Netherlands), preceded by a gurard column. The mobile phase was a mixture of 0.05M phosphate buffer and methanol(90 : 10), the pH being approximately 6.7. Flow rate was 1.4mℓ/min. Detection took place at UV 265nm. Injection

volume was 10 $\mu\ell$

Pharmacokinetic analysis : Pharmacokinetic calculations were performed with the aid of a computer program for non-linear regression analysis(NONLIN). The following kinetic parameters were calculated : area under the plasma conentration-time curve(AUC, mg·h/l), plasma clearance(Cl, l/h/kg), rate constant of elimination(Kel, h^{-1}), distribution volume(Vd, l/kg) and biological half life($t_{1/2}$, h). Pharmacokinetic analyses of N_4-AcSMZ were according to the standard procedure suggested by Baggot.[16] The area under the curve($AUC_{0\rightarrow\infty}$) was calculated using trapezoidal rule up to the last sampling point and extrapolated to calculate the AUC. All parameters were regarded as apparent values because N_4AcSMZ was not administered as a parent drug.

Statistical analysis : Results were expressed as mean ± S.E.M. Means of the values obtained in both sexes were compared by Student's t-test for independent values. The significance level was 5%

Results and Discussion

Pharmacokinetics : The concentration-time curve of SMZ in the rabbit showed a one-compartment open model with first order elimination characteristics(Fig 1). As summarized in Table 1, pharmacokinetic parameters of SMZ indicated that rabbits have a very high capacity to eliminate SMZ, with half-life of only 0.37±0.08h in the female and 0.40±0.08h in the male. The plasma concentration of this parent drug at 4h after administration was 0.09% of the initial concentration in the female and 0.15% in the male. SMZ could not be detected in the plasma at 10h after administration. The half-life of SMZ found in the present experiment is comparable with the value given by Yuan and Fung.[14] They found a half-life

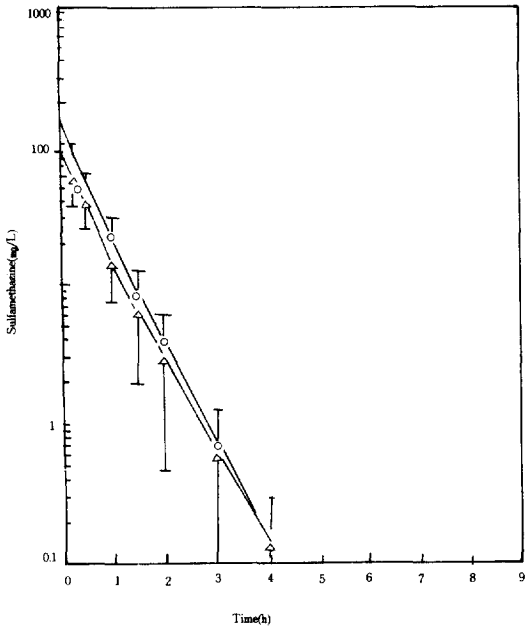

Fig 1. Plasma concentration-time profiles of sulfamethazine following intravenous administration of 35mg/kg of sulfamethazine to male(△) and femal(○) rabbits. Bar indicates S.E.M.

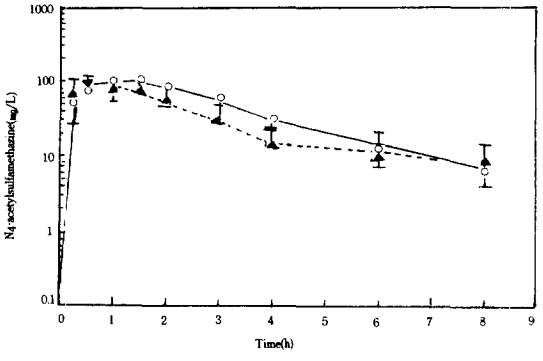

Fig 2. Plasma concentration time profiles of N_4-acetylsul famethazine following intravenous administration of 35mg/kg of sulfamethazine to male(▲) and female(○) rabbits. Bar indicates S.E.M.

Table 1. Pharmacokinetic parameters of SMZ and N_4AcSMZ in the rabbit after i.v. bolus injection of SMZ(35mg/kg) using a computer program(NONLIN) for SMZ and standard methods of Baggot[16] for N_4AcSMZ. Values are means±S.E.M.

	SMZ		N_4AcSMZ	
Parameter	female	male	female	male
Kel(h^{-1})	1.94±0.45	1.81±0.36	0.18±0.03	0.19±0.04
$t_{1/2}$(h)	0.37±0.08	0.40±0.08	3.98±0.70	3.72±2.03
AUC(mg·h/l)	81.20±18.97	55.55±22.57 *	69.26±10.00	53.68±20.85
V_d(l/kg)	0.24±0.06	0.40±0.13 *		
Cl(l/kg/h)	0.46±0.14	0.72±0.28 *		

* Signifcantly different($p<0.05$)

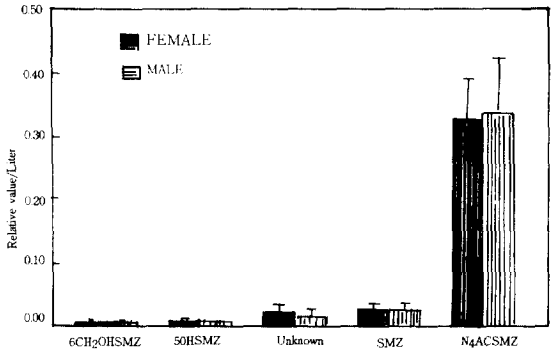

Fig 3. Urinary excretory sulfamethazine metabolites from 24h collected urine after intravenous administration of 35mg/kg of sulfamethazine to male(stipled) and female(black) rabbits. SMZ is short for sulfamethazine. N_4 AcSMZ for N_4-acetylsulfamethazine. $6CH_2OH$SMZ for 6-hydroxymethylsulfamethazine and 5OHSMZ for 5-hydroxysulfamethazine, respectively. Unknown is assumed to be mixture of hydroxy and N_4-acetyl metabolites of SMZ. Vertical lines indicate S.E.M.

of SMZ 1.6±1.3h. This discrepancy could be explainable by their using much higher dosage(200mg/kg) as compared to our 35mg/kg. Different values of half-life in the same animal species of SMZ were reported by many investigators. Wide variation of elimination half of SMZ(4-20h) were shown in ruminants depending on age and dose.[2,17] In calves[17] and in goats[18] capacity-limited SMZ plasma concetration-time curves were obtained at high dose levels of 100mg/kg. As clearly shown in Fig 1, we could not find any difference between two sexes in the elimination curves of SMZ.

N_4AcSMZ concentration in blood was higher than the parent drug from 30min after administration of SMZ. SMZ was rapidly N_4-acetylated to form N_4AcSMZ(Fig 2), which showed a prolonged elimination pattern, longer than SMZ. The peak plasma concentration time of N_4AcSMZ was at about 1h after administration. The 'apparent' pharmacokinetic values were summarized in Table 1. Other pharmacokinetic parameters such as Vd and Cl were not obtained because N_4AcSMZ was not adminstered as a parent drug. There was no sex differences in the elimination of N_4AcSMZ in the rabbit(Fig 2).

Metabolism : In the rabbit, SMZ was mainly acetylated to form N_4AcSMZ but the hydroxylated metabolites could also be detected. As shown in Fig 3, N_4-acetylation was the predominant metabolic pathway in the rabbit. Almost all of the SMZ that was administered was metabolized to N_4AcSMZ. Two hydroxy metabolites(5OHSMZ and $6CH_2OH$SMZ) were also detected in the rabbit urine but in very small amounts, indicating that hydroxylation was relatively unimportant in the rabbit. There was no statistical sex difference in both metabolic pattern and metabolite amount in the 24h collected urine, even though apparently there seemed to appear more N_4AcSMZ in the male rabbit urine than in that of the female. The female showed a slightly higher formation of one of hydroxylation metabolites(5OHSMZ). This, however, represents a minor metabolic pathway. As far as we know, hydroxylation of SMZ has not been described before in the rabbit.

Summary

SMZ is one of the most widely used antibacterial agents in veterinary medicine. It is also used as a growth promotant in many species of domestic animals. There are marked species differences in its metabolism and pharmacokinetics. However, its pharmacokinetic and metabolism in rabbits, which are ragarded not only as good laboratorty animals but also as good economical animals in its own, are lacking. Sex-differences in drug metabolism are well recognized in wide range of animal species including rats. Males are known to be more active than females. It is also know that there are significant differences in the direction of metabolic pathways. But recently, female goats are reported to be more active in the metabolic capacity of SMZ than the other sex by Dutch researchers at Utrecht. Therefore, it is not easy to make general conclusion of having higher SMZ metabolic capacity in the male compared to the opposite sex in every animal species. In this regard, the study on metabolic pattern of SMZ in rabbits, which are regarded as hervivorous, is of interest because the dietary habbits of rabbits are comparable to that of goats. New Zealand White rabbits of each sex were given SMZ(35mg/kg) as a bolus injection into the marginalean vein in order to study its pharmacokinetic profiles(using plasma) anc metabolic pattern(24h urine) as specified in the methods anc materials.

1. In the rabbit, the major metabolic pathway of SMZ was the acetylation(the formation of N_4AcSMZ). There were hydroxylation pathways(5OHSMZ, $6CH_2OH$SMZ) as well, in the metabolism of SMZ in the rabbit, but minor pathways.

2. No sex differences in the metabolic direction of SMZ and its metabolites formation were found from the urinary excreted metabolites of SMZ out of 24h collected urine.

3. The concentration-time curves of SMZ(35mg/kg, iv) in the plasma compartment were fitted to a one-compartment

open model when using a computer program(NONLIN). There was also no difference in the pharmacokinetic pattern of SMZ between two sexes.

4. The emergence of N_4AcSMZ metabolized from SMZ was very fast in the plasma of the rabbit. The elimination of N_4AcSMZ was prolonged as compared to that of the parent drug. We found no sex difference in the elimination pattern of N_4AcSMZ in the rabbit.

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