Original Articles

Tissue distribution of bitespiramycin and spiramycin in rats[1]

Xiang-guo SHI, Yu-ming SUN, Yu-feng ZHANG, Da-fang ZHONG[2]

Laboratory of Drug Metabolism and Pharmacokinetics, Shenyang Pharmaceutical University, Shenyang 110016, China

KEY WORDS bitespiramycin; spiramycin; pharmacokinetics; liquid chromatography; mass spectrum analysis

ABSTRACT

AIM: To investigate the tissue distribution of bitespiramycin (BSPM) and spiramycin (SPM) in rats. **METHODS:** Liquid chromatographic-mass spectrometric assay was applied for the determination of three major components (isovalerylspiramycins, ISV-SPMs) of BSPM and their major active metabolites (SPMs) in rat tissues and plasma after an oral dose of bitespiramycin, as well as SPMs. **RESULTS:** High levels of drug concentrations were observed in most tissues, especially in the liver, stomach, intestine, spleen, lung, womb, and pancreas. BSPM persisted long time in many rat tissues such that the drug concentration in spleen was 69.4 nmol/g at 60 h post-dose and it was still above the minimum inhibitory concentration of many susceptible pathogens. At 2.5 h post-dose, the total concentrations of ISV-SPMs and SPMs achieved in tissues were from 6 to 215 times higher than the corresponding concentrations in plasma. At 2.5 h post-dose, the mean C_t/C_p of BSPM appeared to be 2- or 3-fold those of SPM in most tissues. The tissue to plasma concentration ratios following oral dose of BSPM were higher than those of SPM in most tissues. The drug was not detected in brain and testis after a single dose of BSPM and SPM. **CONCLUSION:** Both BSPM and SPM penetrate into rat tissues well and BSPM has higher tissue affinity than SPM.

INTRODUCTION

Spiramycin (SPM) is a 16-membered macrolide antibiotic produced by *Streptomyces ambofaciens*[1] that consists of three major components: SPMs I, II, and III[2]. SPM is effective *in vivo* for a variety of infections[3], despite the fact that its levels in plasma are often below the MIC for the infective micro-organisms[4]. This can be explained in part by the ability of distribution of SPM into tissues and cells that 10-fold or even higher than the corresponding concentrations in plasma was observed[5,6]. The phenomenon of marked tissue penetration has also been described for the macrolide antibiotic azithromycin[7]. The high intracellular concentrations of these macrolides may explain their good antibiotic activity against susceptible intracellular organisms.

Bitespiramycin (BSPM; shengjimycin) is a group of 4"-acylated SPMs with 4"-isovalerylspiramycins (ISV-SPMs) as the major components, produced by recombinant *Streptomyces spiramyceticus* F21 harboring a 4"-*O*-acyltransferase gene from *Streptomyces mycarofaciens* 1748[8]. BSPM consisted of ISV-SPMs I (7.4%), II (22.5%), and III (37.7%), the other minor components in BSPM include about 7 derivatives of SPM such as butanoylspiramycin and propanoylspiramycin.

BSPM has a similar spectrum of antimicrobial activity to the better known SPM. BSPM was developed

[1] Project supported by the National Natural Science Foundation of China, No 39930180.
[2] Correspondence to Prof Da-fang ZHONG.
Phn/Fax 86-24-2390-2539. E-mail zhongdf@china.com
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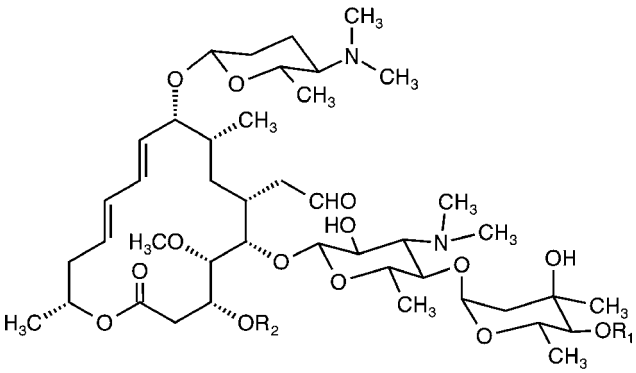
as a novel antibiotic, which was expected to have better pharmacokinetic characteristics than that of SPM. Phase II clinical trial is being undertaken.

In our previous studies[9], SPMs were identified to be the major active metabolites of BSPM. A method using liquid chromatographic-ion trap mass spectrometry for the simultaneous quantitation of ISV-SPMs I, II, III, or SPMs I, II, III in rat plasma and and its application in the pharmacokinetic study were reported[10,11]. In the present investigation, after an oral dose of 80 mg/kg BSPM to rats, ISV-SPMs I, II, III and SPMs I, II, III were determined by the same method, respectively.

The aim of this study was to investigate the tissue distribution of BSPM in comparison with SPM in rat tissues. SPM was chosen because it is widely used in clinical practice and has similar chemical structure with BSPM.



	R_1	R_2
ISV-SPM I	$COCH_2CH(CH_3)_2$	H
ISV-SPM II	$COCH_2CH(CH_3)_2$	$COCH_3$
ISV-SPM III	$COCH_2CH(CH_3)_2$	COC_2H_5
SPM I	H	H
SPM II	H	$COCH_3$
SPM III	H	COC_2H_5

Chemical structures of ISV-SPM I, II, III and SPM I, II, III

MATERIALS AND METHODS

Chemicals and reagents BSPM (Batch No 99-P-M, Content: 953 U/mg) was provided by the Institute of Medical Biotechnology (Beijing, China). SPM (Batch No 20010215, Content: 1016 kU/g with 3 major components spiramycin I, II, and III) was provided by the Chaoyang Pharmaceutical Factory (Liaoning, China). ISV-SPMs I (96.7 %), II (97.0 %), III (96.0 %) and SPMs I (95.0 %), II (98.5 %), III (98.5 %) were isolated by semipreparative HPLC in our laboratory (Isocratic chromatography was performed using either mobile phase of acetonitrile-ammonium 10 mmol/L acetate-acetic acid (35:65:0.5, v:v:v) for the separation of spiramycins I, II, III, and acetylspiramycin III and mobile phase of acetonitrile-ammonium 10 mmol/L acetate-acetic acid (45:55:0.5, v:v:v) for the separation of propanoylspiramycin III, butanoylspiramycin III and isovalerylspiramycins I, II and III). Roxithromycin and azithromycin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Methanol and acetonitrile were of HPLC grade, and other chemicals used were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the study.

Animals and treatment Male Wistar rats weighing 230-270 g (Grade II, Certificate No 042) were purchased from Department of Experimental Animals, Shenyang Pharmaceutical University and housed under isolated and hygienic conditions. They were fasted overnight, with free access to water, for 12-14 h prior to the experiments.

The BSPM or SPM solution was prepared in ethanol-water (1:2, v:v). Rats were given a single oral dose of 80 mg/kg BSPM or SPM. Six rats for each time-point, predose and 2.5, 4.5, 12, 24, and 60 h post-dose, were sacrificed by decapitation after administration of BSPM. Six rats for each time-point, predose and 2.5 and 24 h post-dose, were sacrificed by decapitation when administered SPM. Blood samples (0.5 mL) were collected into heparinized tubes from each rat by puncture of the retro-orbibal sinus. The whole brain, womb, ootheca, testis, pancreas, heart, spleen, stomach, and portions of fat, kidney, intestine, liver, lung, and muscle (skeletal muscle) were quickly removed and weighed, then frozen at -20 ºC.

Blood samples were centrifuged at 2000×*g* for 10 min. Plasma was separated and stored at -20 ºC until assay. All tissues were homogenized with a probe homogenizer (it was washed 5 times with water and acetonitrile before it was used to homogenate another tissue) and 2-fold acetonitrile and 1-fold saline. Tissue homogenate was centrifuged at 2000×*g* for 15 min and supernatant were removed and stored at -20 ºC until assay. For some tissues with high concentrations of drugs, they were diluted by the same blank tissue homogenate.

Sample preparation To a 0.1 mL aliquot of plasma or tissue homogenate were added 200 μL of water, then

50 mL acetonitrile and 50 μL of the internal standard (roxithromycin was used in the determination of ISV-SPMs; azithromycin was used in the determination of SPMs). The samples were vortexed for 15 s. The mixture was adjusted to pH 9-10 with 0.3 mL of Na_2CO_3 0.02 mol/L and extracted with 2 mL of ethyl acetate-isopropanol (95:5, v:v) for 20 min on a roller-shaker. Phase separation was achieved by centrifugation for 10 min at 2000×*g*. The organic layer was removed and evaporated to dryness at 40 ºC under a gentle stream of nitrogen. The residue was dissolved in 100 μL of mobile phase, and vortex mixed. A 20 μL aliquot of the solution was injected into the LC/MS^n system for analysis.

Analytical method A Shimadzu LC-10AD pump (Kyoto, Japan) was used in the experiment. A Finnigan LCQ ion trap mass spectrometer equipped with an electrospray ionization (ESI) source (San Jose, CA) was used for mass analysis and detection.

ISV-SPMs and SPMs concentrations were determined by our previously reported LC/MS^n method[12]. Chromatography was performed on a Kromasil C18 column (150 mm×4.6 mm, 5 μm, Hi-Tech Scientific Instrument Co, Tianjin, China), using mobile phase of acetonitrile-ammonium acetate 10 mmol/L-acetic acid to determine ISV-SPMs (45:55:0.5, v:v:v) and SPMs (35:65:0.5, v:v:v). The flow rate was isocratic at 0.5 mL/min. The column temperature was maintained at 25 ºC. A Finnigan LCQ ion trap mass spectrometer equipped with an ESI source was used for mass analysis and detection. The instrument was operated in the positive ion detection mode, producing positive charged ions in the form of $[M+2H]^{2+}$ and $[M+H]^+$. Double charged ions were trapped and then fragmented by collision induced dissociation. Quantitation was performed using selected reaction monitoring (SRM) mode.

The calibration curves of ISV-SPMs and SPMs were prepared by analyzing spiked plasma and tissue homogenate samples, respectively. Samples at three concentration levels (low, medium, and high concentrations) were used as QC samples and analyzed by LC/MS^n system.

During prestudy validation[14], the calibration curves were defined in three runs based on triplicate assays of the spiked plasma and tissue homogenate samples (heart and liver) respectively. QC samples were determined in replicates (n=18) on the same run. Overall assay performance was assessed by calculating the accuracy and intra- and inter-run precision of QC samples analyzed. During routine analysis each analytical run included a set of calibration samples, a set of QC samples in duplicate and unknowns.

RESULTS

Method validation The linear calibration curves were obtained in the concentration ranges of 4-200 mg/L for ISV-SPM I and SPM I, 12-600 mg/L for ISV-SPM II and SPM II, 18-900 mg/L for ISV-SPM III and SPM III with a correlation coefficient of >0.993 using every control tissue homogenate, respectively. Tab 1 shows the intra- and inter-run precision and accuracy for ISV-SPMs and SPMs from the QC samples in the homogenate samples of rat hearts. The method was applied for the determination of ISV-SPMs and SPMs in the plasma and tissue homogenate samples. The concentrations of ISV-SPMs and SPMs represent the total concentrations of ISV-SPMs I, II, III, and SPMs I, II, III, respectively.

Tissue distribution of BSPM ISV-SPMs (the total of ISV-SPMs I, II, III) were present in measurable amounts in all tissues except in brain and testis.

Tab 1. Summary of precision and accuracy from QC samples of rat heart extracts (in prestudy validation, n=18)

Analyte	Intra-run RSD/%	Inter-run RSD/%	Relative error/%	Typical equation of calibration curve
ISV-SPM I	7.5	6.5	12.1	$y=1.206\times10^{-3}+7.388\times10^{-4}\,x$, $r=0.9939$
ISV-SPM II	5.6	4.8	6.5	$y=4.809\times10^{-3}+6.471\times10^{-4}\,x$, $r=0.9945$
ISV-SPM III	13.6	6.4	9.1	$y=6.169\times10^{-3}+6.028\times10^{-4}\,x$, $r=0.9981$
SPM I	6.0	4.9	4.9	$y=1.181\times10^{-3}+4.248\times10^{-4}\,x$, $r=0.9937$
SPM II	6.1	11.2	7.8	$y=1.258\times10^{-2}+1.895\times10^{-4}\,x$, $r=0.9952$
SPM III	3.6	6.9	8.1	$y=4.633\times10^{-3}+6.362\times10^{-4}\,x$, $r=0.9941$

Maximum levels of ISV-SPMs concentrations were seen in most tissues by 2.5 h, but the peak concentrations in the lung, spleen, and pancreas were observed at 12 h or 4.5 h. The exposure in plasma was very low (0.03 μmol/L at 2.5 h post-dose). By 60 h post-dose, concentrations of ISV-SPMs in most tissues were still above the limits of quantitation (Fig 1).

Higher levels of SPMs (the total of SPMs I, II, and III) concentrations were observed in most tissues and persisted longer time than those of ISV-SPMs in these tissues, with the exception of stomach and intestine, where concentrations and elimination rate were similar to those of ISV-SPMs. By 60 h post-dose, concentrations of SPMs in most tissues were much higher than the limits of quantitation. Maximum levels of SPMs were observed in womb, lung, spleen, and ootheca at 12 h or 4.5 h post-dose, there were marked accumulation of SPMs due to slow elimination from these tissues. The concentrations of SPMs in tissues and plasma following oral administration of 80 mg/kg BSPM are shown in Fig 2.

Comparative tissue distribution of BSPM and SPM After a single dose (80 mg/kg) of SPM, SPMs were present in measurable amounts in all tissues except that in brain and testis. The concentrations of SPMs in the tissues and plasma are summarized in Tab 2. The total concentrations of ISV-SPM and SPM at 2.5 h and 24 h after an oral dose of 80 mg/kg BSPM are listed on the left two columns, while the concentrations of SPM at 2.5 h and 24 h after the same oral dose of SPM are listed on the right two colunms. The tissue distribution of the two drugs in rats is similar, but obviously higher tissue concentrations are observed after a single dose of BSPM.

The tissue to plasma concentration ratio (C_t/C_p) following an oral dose of the two drugs are summarized in Tab 3. At 2.5 h post-dose, the total concentrations of ISV-SPMs and SPMs achieved in tissues were 6-215 folds higher than the corresponding concentrations in plasma. The distribution to tissues was higher when given BSPM in comparison with SPM. At 2.5 h post-dose, the mean C_t/C_p of BSPM appeared to be 2- or 3-fold those of SPM in most tissues. After a single dose of BSPM or SPM, there were large inter-individual variations observed in the plasma and tissue concentrations.

Tab 2. The tissue concentrations following oral administration of BSPM and SPM (80 mg/kg) in rats. *n*=6. Mean±SD.

Tissue	Concentration/μmol·kg^{-1} or μmol·L^{-1}			
	BSPM		SPM	
	2.5 h[1)]	24 h[1)]	2.5 h	24 h
Heart	22.2±9.6	5.7±1.1	15±6.9	7.9±1.9
Liver	138.5±34.9	64.3±6.2	71.7±47.7	33.4±16.8
Spleen	82.9±13.8	110.4±21.5	45.3±20.3	78.9±31.6
Lung	49.4±11.3	39.5±9.5	20.7±10.0	27.6±11.6
Kidney	32.1±4.4	9.2±2.5	13.3±3.8	15.2±5.3
Fat	13.1±2.3	4.9±1.8	2.3±0.6	2.2±0.6
Pancreas	38.5±15.3	31.8±11.3	43.2±11.9	25.0±16.4
Intestine	74.9±28.8	19.8±3.8	43.5±15.2	12.4±3.8
Stomach	92.9±35.0	19.8±3.9	31.2±9.5	10.9±4.4
Muscle	4.6±2.2	3.4±0.8	3.1±1.8	2.3±1.3
Womb	46.7±21.1	37.4±14.8	6.4±2.6	14.3±7.0
Ootheca	29.9±14.3	33.6±6.3	13.1±6.2	14.7±6.9
Plasma	0.6±0.3	0.20±0.03	0.9±0.3	0.10±0.03

[1)] The total concentrations of ISV-SPMs and SPMs in rat tissues.

DISCUSSION

The gastrointestinal tract did not exhibit the highest exposures (lower than liver and spleen), which should be attributed to the rapid and complete absorption in the gastrointestinal tract and rapid uptake of unchanged

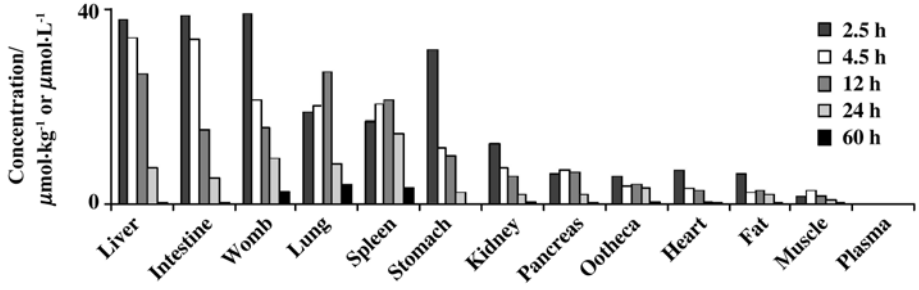


Fig 1. The mean concentrations of ISV-SPMs in the plasma and tissues at 2.5, 4.5, 12, 24, and 60 h after a single oral dose of 80 mg/kg BSPM. *n*=6.

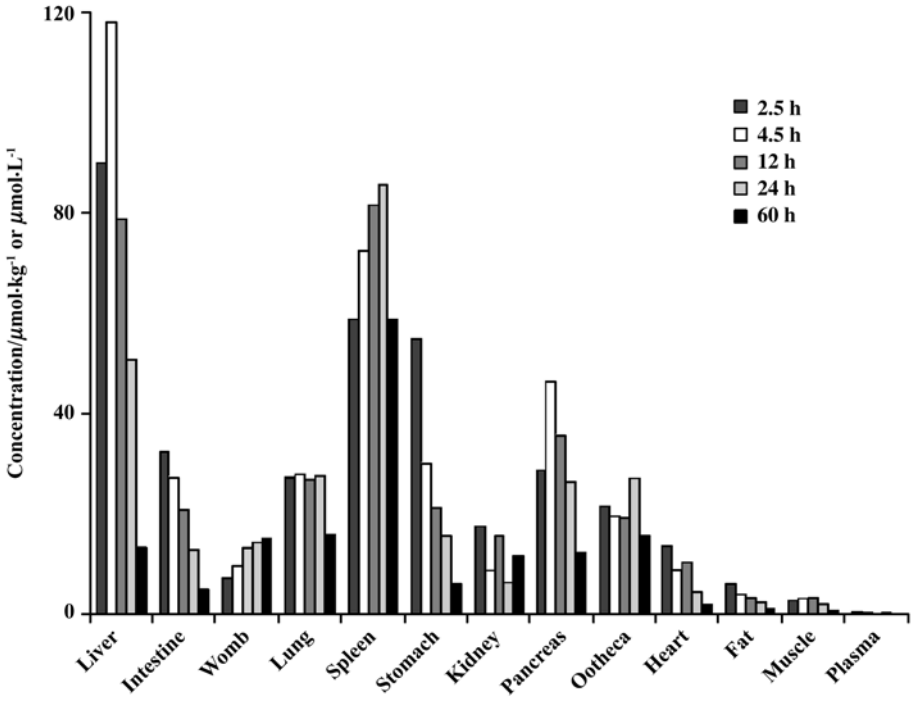


Fig 2. The mean concentrations of SPMs in the plasma and tissues at 2.5, 4.5, 12, 24, and 60 h after a single oral dose of 80 mg/kg BSPM. *n*=6.

Tab 3. Comparative tissue distribution following oral administration of BSPM and SPM (80 mg/kg) respectively. *n=6.*

Tissue	C_t/C_p [1]				C_{BSPM}/C_{SPM} [2]	
	2.5 h		24 h		2.5 h	24 h
	BSPM	SPM	BSPM	SPM		
Heart	35	17	35	68	1.5	0.7
Liver	216	85	402	285	1.9	1.9
Spleen	134	54	690	674	1.9	1.4
Lung	77	24	247	236	2.4	1.4
Kidney	50	16	58	130	2.4	0.6
Fat	20	3	30	18	5.7	2.3
Pancreas	60	51	199	213	0.9	1.3
Intestine	117	51	124	106	1.7	1.6
Stomach	145	37	124	93	3.0	1.8
Muscle	7	4	21	20	1.5	1.5
Womb	73	8	233	122	7.3	2.6
Ootheca	47	16	212	126	2.3	2.3
Plasma	1	1	1	1	0.8	1.4

[1] Tissue to plasma concentration ratio.

[2] C_{BSPM} and C_{SPM} represent the mean concentrations in tissues following oral dose of BSPM and SPM respectively.

form and SPMs by tissues from the circulation, a property similar with that of SPM[12]. ISV-SPMs existed in comparative concentrations with SPMs in intestine and stomach, whereas plasma levels of the former were much lower than the latter, suggesting that most of BSPM should be extensively metabolized to SPM due to first-pass metabolism.

BSPM is well distributed to tissues as indicated by the high C_t/C_p values in the tissue distribution study. Concentrations of ISV-SPMs reached their peak values in most tissues at about 2.5 h, whereas, concentrations of SPMs reached their peak values in most tissues at about 4.5-24 h, in some tissues, such as in womb, even at 60 h. The total concentrations of ISV-SPMs and SPMs were still above the reported MIC for most sensitive organisms[13] in most tissues at 60 h post-dose, indicating that there was still antibiotic activity in the animals.

The average plasma concentration of the total of ISV-SPMs and SPMs at 2.5 h after oral dose of BSPM was lower than that of the SPMs after oral dose of SPM. But at 24 h post-dose, the average total plasma concentration was higher than that after oral dose of SPM. Compared with SPM, slower uptake of BSPM by tissues from plasma and slower redistribution from tissues to plasma may be assumed for the above phenomenon.

The drug concentrations following oral administration of BSPM or SPM were below the limits of quantitation in brain and testis, which was consistent

with previous report[14]. However, high levels of drug concentrations were observed in the womb and ootheca. It suggested that certain degree gender difference existed in the distribution of BSPM and SPM. SPM disposition in genital tract in the ewe have been investigated because SPM is potentially active against most of the microorganisms isolated from secretions of infective genital tracts[15,16]. After a single dose of BSPM, the total concentration of ISV-SPM and SPM in womb was higher than that after the same oral dose of SPM at 2.5 h or 24 h. Based on these results, BSPM may have better effect on the womb and genital tracts infections.

Pharmacodynamic models and susceptibility breakpoints derived from studies with other classes of drugs, such as the beta-lactams and aminoglycosides, do not adequately explain the clinical utility of antibacterial agents that achieve high intracellular concentrations. In the present investigation, ISV-SPMs and SPMs were found at low levels in rat plasma, but they were found at high concentrations and achieved high C_t/C_p in most tissues. The similar characters were also observed in azithromycin and clarithromycin[7].

Both BSPM and SPM are rapidly and widely distributed throughout the body and achieved high C_t/C_p ratios in most tissues in rat. As concentrations in tissues corresponded to the potency of SPM derivatives, and higher C_t/C_p ratios were observed for BSPM compared with SPM, we predicted that less dose of BSPM or longer dose periods could be used in clinical therapy than those of SPM.

REFERENCES

1 Schauner C, Dary A, Lebrihi A, Leblond P, Decaris B, Germain P. Modulation of lipid metabolism and spiramycin biosynthesis in *Streptomyces ambofaciens* unstable mutants. Appl Environ Microbiol 1999; 65: 2730-7.

2 Ramu K, Shringarpure S, Cooperwood S, Beagle JM, Williams JS. ^{1}H-NMR and ^{13}C-NMR spectral assignments of spiramycins I and III. Pharm Res 1994; 11: 458-65.

3 Rubinstein E, Keller N. Spiramycin renaissance. J Antimicrob Chemother 1998; 42: 572-6.

4 Smith CR. The spiramycin paradox. J Antimicrob Chemother 1988; 22 (Suppl B): 141-4.

5 Harf R, Panteix G, Desnottes JF, Diallo N, Leclercq M. Spiramycin uptake by alveolar macrophages. J Antimicrob Chemother 1988; 22 (Suppl B): 135-40.

6 Kitzis M, Desnottes JF, Brunel D, Giudicelli A, Jacotot F, Andreassian B. Spiramycin concentrations in lung tissue. J Antimicrob Chemother 1988; 22 (Suppl B): 123-6.

7 Amsden GW. Advanced-generation macrolides: tissue-directed antibiotics. Int J Antimicrob Agents 2001; 18 (Suppl 1): S11-5.

8 Shang GD, Dai JL, Wang YG. Construction and physiological studies on a stable bioengineered strain of shengjimycin. J Antibiot (Tokyo) 2001; 54: 66-73.

9 Shi XG, Zhong DF, Sun YZ, Zhang YF. Metabolites of a novel antibiotic bitespiramycin in rat urine and bile. Chin Chem Lett 2004: 15: 431-4.

10 Zhong DF, Shi XG, Sun L, Chen XY. Determination of three major components of bitespiramycin and their major active metabolites in rat plasma by liquid chromatography-ion trap mass spectrometry. J Chromatogr B 2003; 791: 45-53.

11 Shi XG, Zhong DF, Sun L. Pharmacokinetics of a novel antibiotic bitespiramycin in rats. Asian J Drug Metab Pharmacokinet 2003; 3: 134-7.

12 Osono T, Umezawa H. Pharmacokinetics of macrolides, lincosamides and streptogramins. J Antimicrob Chemother 1985; 16 (Suppl A): 151-66.

13 Hardy DJ, Hensey DM, Beyer JM, Vojtko C, McDonald EJ, Fernandes P. Comparative *in vitro* activities of new 14-, 15-, and 16-membered macrolides. Antimicrob Agents Chemother 1988; 32: 1710-9.

14 Schoondermark-Van de Ven E, Glama J, Camps W, Vree T, Russel F, Meuwissen J, *et al*. Pharmacokinetics of spiramycin in the Rhesus monkey: transplacental passage and distribution in tissue in the fetus. Antimicrob Agents Chemother 1994; 38: 1922-9.

15 Cester CC, Laurentie MP, Garcia-Villar R, Toutain PL. Spiramycin concentrations in plasma and genital-tract secretions after intravenous administration in the ewe. J Vet Pharmacol Ther 1990; 13: 7-14.

16 Cester CC, Dubech N, Toutain PL. Effect of sexual steroid hormones on spiramycin disposition in genital tract secretions of the ewe. J Pharm Sci 1992; 81: 33-6.