Chinese Chemical Letters Vol. 15, No. 4, pp 431-434, 2004
http://www.imm.ac.cn/journal/ccl.html

Metabolites of A Novel Antibiotic Bitespiramycin in Rat Urine and Bile

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Abstract: A sensitive analytical method to identify active metabolites of bitespiramycin in rat urine and bile was developed by liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS^n). Bitespiramycin and its major active metabolites in rat urine and bile were isolated and identified as M1 serial (spiramycin I, II, III), M2 serial (platenomycin A1, josamycin and leucomycin A1) and M3 serial (deisovalerylplatenomycin A1, deisovaleryl-josamycin, deisovalerylleucomycin A1).

Keywords: Spiramycin, bitespiramycin, metabolites.

Bitespiramycin (Shengjimycin) was developed by Chinese Academy of Medical Sciences. It is a group of 4''-acylated spiramycins with 4''-isovalerylspiramycins as the major components, produced by recombinant *Streptomyces spiramyceticus* F21 harboring a 4''-O-acyltransferase gene[1,2]. Phase I clinical research of bitespiramycin is undergoing.

Bitespiramycin was proved to have similar antibiotic activity with spiramycin *in vitro*. But it has better pharmacokinetic behavior than spiramycin *in vivo*[3]. The metabolites of bitespiramycin in rat urine and bile were analyzed and identified in the present paper, for further investigating of their antibacterial activities.

Experimental

The Finnigan LCQ ion trap mass spectrometer (San Jose, CA, USA) with electrospray interface (ESI) was used for the determination of bitespiramycin and its metabolites. Four rats weighing approximately 200 g were orally administered bitespiramycin 80 mg/kg. The urine and bile were collected at 0-12 h and 12-24 h postdose and filtered before they were loaded onto solid phase extraction (SPE) C_{18} bond elut columns, which were preconditioned with 3 mL of acetonitrile and then 3 mL of water, respectively. Samples were eluted with 2 mL acetonitrile and the elutes were injected into the LC/MS^n system for analysis. Samples were analyzed on a Kromasil C_{18} column (200 mm × 4.6

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Figure 1 Full scan MS^2 chromatograms of isovalerylspiramycins and their metabolites in rat urine

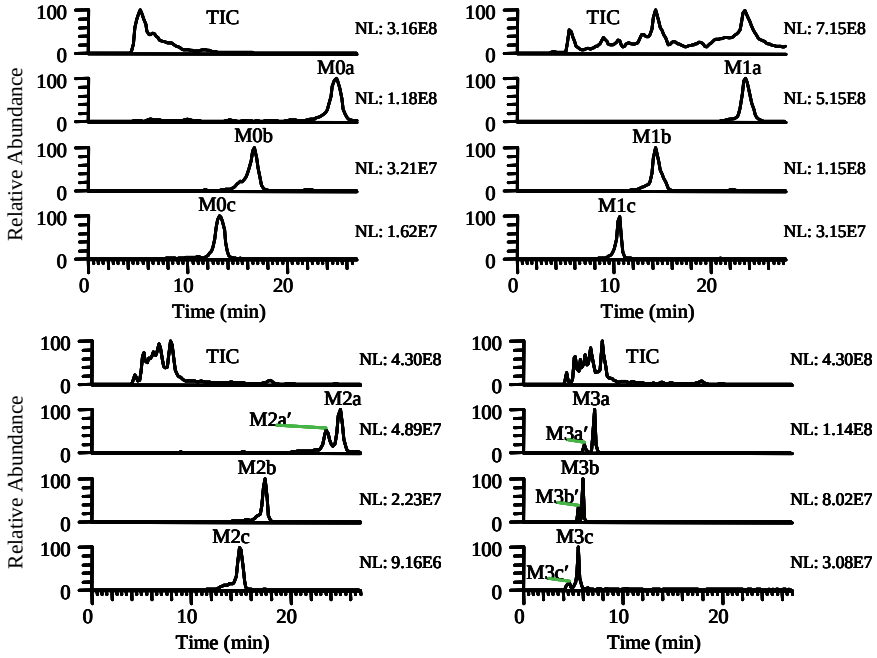
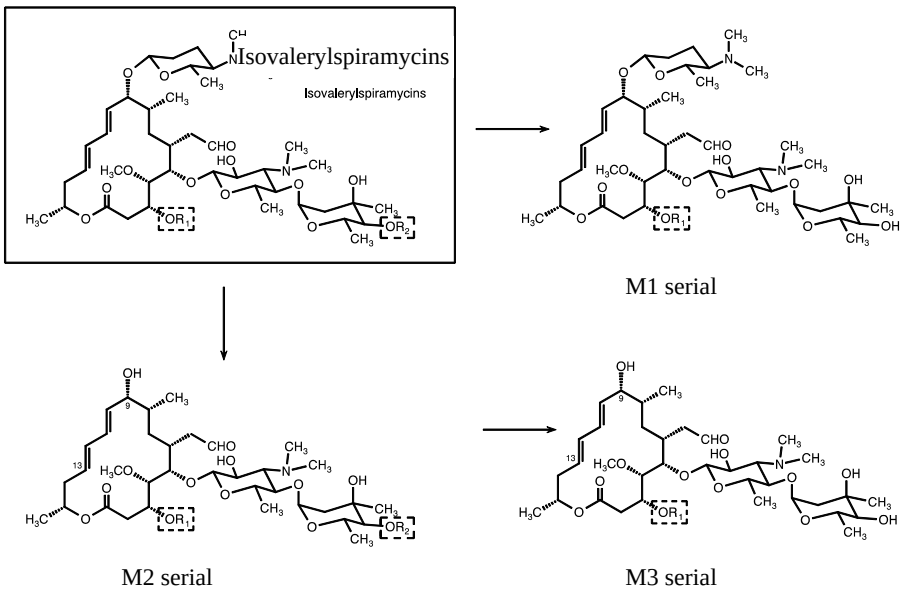


Figure 2 Proposed metabolic pathways of bitespiramycin in rat



mm, 5 μm particle size, Hi-Tech Scientific Instrument Co., Tianjin, China). The mobile phase consisted of acetonitrile:10 mmol/L ammonium acetate:acetic acid (25:75:0.25, for M1 serial; 35:65:0.5, for isovalerylspiramycins; 45:55:0.5, for M2 and M3 serials, v/v/v). The flow rate was 0.5 $mL \cdot min^{-1}$. The column temperature was maintained at 25 °C.

Isovalerylspiramycin I, II, III, spiramycin I, II, III and platenomycin A1 were isolated by semipreparative HPLC. Josamycin was purchased from Yamanouchi Co. and leucomycin A1 was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Table 1 The protonated molecules and MS^2 fragment ions of isovalerylspiramycins and their metabolites

Metabolites	*m/z*	MS^2	Identify	R_1	R_2
M0a	983	824, 755, 596	Isovalerylspiramycin III	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
M0b	969	810, 741, 582	Isovalerylspiramycin II	$COCH_3$	$COCH_2CH(CH_3)_2$
M0c	927	768, 699, 540	Isovalerylspiramycin I	H	$COCH_2CH(CH_3)_2$
M1a	899	755, 740, 596	Spiramycin III	$COCH_2CH_3$	H
M1b	885	741, 726, 582	Spiramycin II	$COCH_3$	H
M1c	843	699, 684, 540	Spiramycin I	H	H
M2a	842	614	Platenomycin A1	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
M2b	828	600	Josamycin	$COCH_3$	$COCH_2CH(CH_3)_2$
M2c	786	558	Leucomycin A1	H	$COCH_2CH(CH_3)_2$
M3a	758	614	Deisovalerylplatenomycin A1	$COCH_2CH_3$	H
M3b	744	600	Deisovaleryljosamycin	$COCH_3$	H
M3c	702	558	Deisovalerylleucomycin A1	H	H

Results and Discussion

Compared with blank urine and bile of rats, isovalerylspiramycins and their metabolites were found in the urine and bile of rats following oral administration of 80 mg/kg bitespiramycin (**Figure 1)**. Two serials of 16-membered macrolide antibiotics (M1 serial and M2 serial), which have been widely used in clinical therapy for decades, were identified. Isovalerylspiramycins were metabolized to M1 serial with the loss of 4"-isovaleryl group under the effect of esterase *in vivo*. Proposed metabolic pathways of bitespiramycin in rat were described as **Figure 2**. The protonated molecules and MS^2 fragment ions of isovalerylspiramycins and their metabolites were listed in **Table 1**. M0, M1 and M2 serials were identified by comparing chromatographic and mass spectrometric behaviors with those of corresponding reference substances, respectively.

Spiramycins were the major metabolites of bitespiramycin. The metabolic pathway was similar with that of acetylspiramycin in rats[4]. The fact, that the antibiotic activities of isovalerylspiramycins were similar to spiramycins *in vitro*, proved that the loss of 4"-isovaleryl group has no large effect on antibiotic activity. So it was concluded that M3 serial may have similar antibiotic activity with M2 serial.

The metabolic pathway from isovalerylspiramycins to M2 serial was induced under the effect of acidic environment in rat stomach. It has been proved by our experiment *in vitro*. In acidic environment (pH < 2), the forosamine group was hydrolyzed and their degradation products were platenomycin A1, josamycin and leucomycin A1, respectively. This metabolic pathway has not been reported in the literature.

In acidic environment, M2 and M3 serials may transform to their isomers (hydroxyl group at position 9 drifted to position 13)[5]. Under the above chromatographic conditions, M2a′, M3a′, M3b′ and M3c′ were separated and their MS^2 fragments were the same with those of M2a, M3a, M3b and M3c, respectively.

Acknowledgments

This research project (39930180) was supported by the National Natural Science Foundation of China.

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Received 26 March, 2003