ABSORPTION, TISSUE DISTRIBUTION AND EXCRETION OF 19-DEFORMYLDESMYCOSIN DERIVATIVES, NEW 16-MEMBERED MACROLIDES, IN MICE

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19-Deformyl derivatives of desmycosin[1,2] are new 16-membered macrolide antibiotics lacking an aldehyde group with strong activity against Gram-positive aerobes, anaerobes and mycoplasmas.[3] Among the derivatives, 19-deformyl-4'-deoxydesmycosin (TMC-016) has been shown to be 4- to 7-fold more potent than erythromycin against Gram-positive bacterial infections in experimental animals.[3]

In this study we investigated the absorption, tissue distribution and excretion of the derivatives, 19-deformyldesmycosin (TMC-014), 19-deformyl-4''-deoxydesmycosin (TMC-015) and 19-deformyl-4'-deoxydesmycosin (TMC-016) in mice, and compared the results with those for the 19-formyl derivative, 4'-deoxydesmycosin.[4]

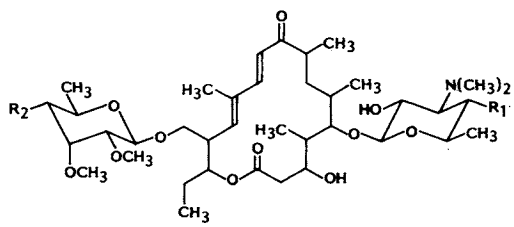
TMC-014, TMC-015, TMC-016 and 4'-deoxydesmycosin were prepared at Toyo Jozo Laboratories, Shizuoka, Japan. A solution (2 mg/ml) of each drug for iv infusion was obtained by dissolving 20 mg of the drug in 10 ml of sterile saline. A solution of each drug for po administration was obtained by dissolving 20 mg of the drug in 10 ml of citric acid-glycine solution (pH 4.0). ICR male mice (6 weeks old, Shizuoka Laboratory Animal Center, Shizuoka, Japan) were used. Single po and iv doses (20 mg/kg) of the drugs were administered to fasting (18 hours) mice for pharmacokinetic studies of plasma and tissues.

Mouse blood samples were collected in a heparinized syringe by cardiac puncture under anesthesia, and centrifuged to collect the plasma after the addition of diisopropyl fluorophosphate (DFP) to 0.3 mM final. Blood samples were taken at 5, 15, 30 and 60 minutes after iv administration and at 0.25, 0.5, 1 and 2 hours after po administration of the drug. Three mice were used at each time point. Tissue samples were taken at these times to determine the tissue distribution of the drug. Tissues were processed as follows: The tissues were removed, weighed and homogenized in 3 volumes of 1/15 M phosphate buffer (pH 8.0) with a Polytron homogenizer. The resulting samples were centrifuged (3,000 rpm, 10 minutes) after the addition of DFP and the supernatants thus obtained were kept frozen at −20°C until analyzed. During periods of 0 to 24, 24 to 48 hours, urine and feces were collected in metabolic cages. The urine samples were diluted with 1/15 M phosphate buffer (pH 8.0) after the addition of DFP. The feces were homogenized in 10 volumes of the same buffer with a Polytron homogenizer and centrifuged after the addition of DFP.

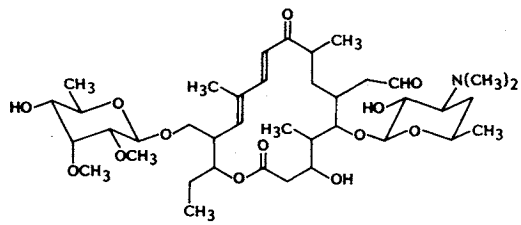
The bioactivity in the samples was determined by an agar-well method using *Micrococcus luteus* ATCC 9341 as the test organism, in Trypto Soy Agar (pH 8.0, Eiken, Japan). Standard curves for the drugs were prepared with plasma or tissue homogenates from control mice.

The tissue and plasma concentrations of the drugs were determined by bioassay in mice after iv administration at a 20-mg/kg dose and the results are shown in Table 1. The concentrations of these compounds in plasma were 7.0 to 9.6 μg/ml 5 minutes after administration and were reduced to 0.8 to 2.2 μg/ml after 1 hour. These compounds were rapidly distributed to all tissues and showed excellent

Fig. 1. Structure of 19-deformyl derivatives of desmycosin and 4'-deoxydesmycosin.



TMC-014 $R_1=R_2=OH$
TMC-015 $R_1=OH$ $R_2=H$
TMC-016 $R_1=H$ $R_2=OH$



4'-Deoxydesmycosin

Table 1. Pharmacokinetics of 19-deformyl derivatives and 4′-deoxydesmycosin in mice after iv administration.

Compound	TMC-014			TMC-015		
	$C_{5\,minutes}$[a]	$T_{1/2}$ (hour)	AUC[b]	$C_{5\,minutes}$	$T_{1/2}$ (hour)	AUC
Plasma	8.5	0.26	4.1	7.0	0.34	4.2
Liver	85.7	0.31	39.5	35.8	0.49	24.7
Kidney	61.2	0.63	56.6	126.5	0.40	56.3
Heart	27.5	0.33	10.8	12.5	0.32	5.4
Lung	26.1	0.76	27.4	68.1	0.71	63.0
Spleen	26.2	0.52	19.0	19.4	0.55	18.5
Skin	6.6	1.04	10.3	5.8	0.76	7.7

Compound	TMC-016			4′-Deoxydesmycosin		
	$C_{5\,minutes}$	$T_{1/2}$ (hour)	AUC	$C_{5\,minutes}$	$T_{1/2}$ (hour)	AUC
Plasma	9.4	0.42	6.7	9.6	0.34	4.6
Liver	29.3	0.64	27.8	133.4	0.26	48.8
Kidney	21.0	0.73	18.6	36.2	0.46	24.8
Heart	8.9	0.47	5.9	13.1	0.35	5.1
Lung	19.9	1.09	31.8	14.7	0.61	9.3
Spleen	15.7	0.77	20.3	27.0	0.66	24.0
Skin	5.1	1.21	9.8	4.5	0.57	3.8

[a] Concentration at first sampling point (μg/ml or μg/g).
[b] μg·hour/ml or μg·hour/g.

penetration in the lung, liver, spleen and kidney. In the lung, TMC-015 showed the best penetration (AUC: 63.0 μg·hour/g) among the drugs tested; on the other hand TMC-016 showed a prolonged $T_{1/2}$ (1.09 hours). Whereas the administration of TMC-015 resulted in the highest antibiotic concentrations in the kidney, 4′-deoxydesmycosin produced the highest level in the liver. However, both derivatives were rapidly eliminated from these organs. The levels in skin were gradually elevated to achieve the maximum concentration (Cmax), and the high levels were long-lasting.

The 19-deformyl derivatives, TMC-014, TMC-015 and TMC-016, were rapidly absorbed when administered orally and were demonstrated tissue distributions comparable to that following iv administration. As shown in Table 2, the Cmax of TMC-014, TMC-016 and TMC-015 in plasma after administration of 20 mg of the drug per kg was 3.2, 2.2 and 2.0 μg/ml, respectively. TMC-014 showed the highest levels in almost all tissues and the levels in the lung were 8.5 μg/g, indicating excellent penetration. Although the tissue levels of TMC-015 were rather low, the levels in the lung were exceptionally high, a Cmax of 10.1 μg/g and an AUC ratio (tissue/plasma) of 11.0.

On the other hand, the 19-formyl derivative, 4′-deoxydesmycosin, showed very poor absorption when orally administered. The plasma level 2 hours after administration was only 0.1 μg/ml and the tissue levels were also low even in the liver.

The bioavailability (as estimated by po/iv AUC ratios) of TMC-014 and TMC-016 was excellent (105 and 81%, respectively) and that of TMC-015 was rather low (54%).

The cumulative excretion data in urine and feces are shown in Table 3. Over a 48-hour period, 37.0, 21.0 and 49.1% of the iv dose of TMC-014, TMC-015 and TMC-016 was excreted as microbiologically active compound. Similarly, 33.2% of the iv dose of 4′-deoxydesmycosin was excreted.

4′-Deoxydesmycosin possessing an aldehyde function and 19-deformyl derivatives of desmycosin, TMC-014, TMC-015 and TMC-016, penetrated well into all tissues after iv administration. However, 4′-deoxydesmycosin, as well as desmycosin and tylosin[5] showed very poor absorption, whereas all the deformyl derivatives were well absorbed and rapidly distributed to all tissues when orally administered. This finding suggests that the greatly increased efficacy of the 19-deformyl compounds[3] when administered orally is caused mainly by their increased absorption.

In the studies with iv administration of the drugs, 4′-deoxydesmycosin was rapidly distributed to all tissues, especially the liver; however, its elimination

Table 2. Pharmacokinetics of 19-deformyl derivatives and 4'-deoxydesmycosin in mice after po administration.

Compound	TMC-014			TMC-015		
	Cmax[a]	AUC[b]	AUC ratio[c]	Cmax	AUC	AUC ratio
Plasma	3.2	4.3		2.0	2.2	
Liver	57.4	51.2	11.8	19.9	20.2	9.0
Kidney	40.8	65.6	15.2	14.3	26.9	12.0
Heart	6.4	18.3	4.2	2.6	4.0	1.8
Lung	8.5	37.4	8.6	10.1	24.6	11.0
Spleen	10.6	43.2	10.0	4.5	11.7	5.2
Skin	2.3	16.4	3.8	1.5	4.1	1.8

Compound	TMC-016			4'-Deoxydesmycosin		
	Cmax	AUC	AUC ratio	Cmax	AUC	AUC ratio
Plasma	2.2	5.4		0.1	0.2	
Liver	23.7	33.0	6.1	2.0	3.4	15.3
Kidney	13.1	65.4	12.1	0.9	1.3	5.9
Heart	4.0	6.7	1.2	ND	ND	ND
Lung	6.0	34.2	6.3	0.4	0.5	2.4
Spleen	8.7	17.6	3.3	0.6	1.0	4.3
Skin	1.9	4.8	0.9	0.1	0.2	0.9

[a] μg/ml or μg/g. [b] μg·hour/ml or μg·hour/g. [c] tissue/plasma.
ND: None detected.

Table 3. Urinary and fecal recovery of 19-deformyl derivatives and 4'-deoxydesmycosin in mice.

Compound	Time (hours)	Recovery (% of dose) in		
		Urine	Feces	Urine+feces
TMC-014	0~24	14.8	17.3	32.1
	24~48	0.7	4.2	4.9
	0~48	15.5	21.4	37.0
TMC-015	0~24	9.3	10.6	19.9
	24~48	0.7	0.4	1.1
	0~48	10.0	11.0	21.0
TMC-016	0~24	32.3	15.3	47.6
	24~48	0.8	0.7	1.5
	0~48	33.1	16.0	49.1
4'-Deoxy-desmycosin	0~24	3.0	29.2	32.2
	24~48	0.1	0.9	1.0
	0~48	3.1	30.1	33.2

(including metabolism) from the tissues was faster than that of 19-deformyl derivatives. Among the 19-deformyl derivatives, TMC-016 showed the most prolonged maintenance in all tissues, suggesting a favorable therapeutic effect.

TMC-015 is the deoxygenated derivative of TMC-014 at the C-4″ position. Deoxygenation of the hydroxyl group in mycinose resulted in characteristic changes in the properties of TMC-014. TMC-015 penetrated the kidney and lung in very high concentrations, however, it was excreted rapidly from the kidney. The levels of TMC-015 in the lung were also high when it was administered orally and almost equal to that of clarithromycin, which has been reported to have excellent ability to penetrate the lung.[6] However, the bioavailability of TMC-015 was lower than that of TMC-014 and TMC-016.

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