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Residues of Macrolide Antibiotic Sedecamycin and Its Major Metabolites in Swine Blood and Tissues

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Pigs were fed a diet containing 50–500 ppm of the macrolide antibiotic sedecamycin for 14 or 28 days starting at 6 or 10 weeks of age. Tissues were collected to determine sedecamycin and its three biologically active metabolites at designated times after the drug was withdrawn. Of the edible tissues, liver retained the highest metabolite concentrations at 2 h after withdrawal. Blood, muscle, and fat showed no drug residue at any time even at the 500 ppm level of medication. The small intestine showed higher concentrations of the unchanged drug and metabolites than the liver. All compounds disappeared rapidly from all the tissues and were not detected in any tissue at 1 day after withdrawal and thereafter.

Sedecamycin (I, Figure 1), one of the lankacidin group of antibiotics produced by *Streptomyces rochei* var. volubilis (Harada et al., 1969), possesses preeminent activity against *Treponema hyodysenteriae*, the pathogenic organism for swine dysentery (Narukawa et al., 1984; Yamazaki et al., 1986), in addition to antibacterial activities against gram-positive organisms (Tsuchiya et al., 1971). In Japan, I has been used at a concentration of 25–75 ppm in feed to treat swine dysentery since it was approved in 1985. Before approval was granted, it was obligatory to examine the residue of I in swine tissues to determine the withdrawal period before slaughter.

When [^{14}C]I was administered orally to swine, 61% of the radioactivity was excreted as approximately 20 lipophilic and some polar metabolites in the bile, urine, and feces within 48 h (Okada et al., 1984). Among the lipophilic metabolites, lankacidin C (II), lankacidinol A (III), and lankacidinol (IV) exhibited antibacterial activity against *T. hyodysenteriae* (Narukawa et al., 1984). As most lipophilic metabolites showed polarities between those of I and IV, these four compounds were considered to represent the tissue affinity of the other metabolites. For these reasons, I–IV were monitored in swine tissues to know the residual profile of I after it was administered continuously in feed.

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MATERIALS AND METHODS

Chemicals. Sedecamycin used in these residue studies was technical grade of 97.8% purity; it was produced in the Applied Microbiology Laboratories of Takeda Chemical Industries. Analytical standard compounds were prepared in the laboratories and their purities were as follows: I, 99.2%; II, 99.7%; III, 94.2%; IV, 93.1%.

Hexane and 2-propanol were HPLC grade from Wako Pure Chemical Industries Ltd., Osaka. Silica gel 60 (70–230 mesh, Merck) and Florisil (100–200 mesh, Floridin Co.) were dried 6 h at 130 °C before use. The other reagents were reagent grade from Wako Pure Chemical Industries.

Animal Treatment. Two experiments were carried out. In experiment 1, six Landrace pigs (five castrated and one female) aged 6 weeks and nine Landrace pigs (five castrated and four female) aged 10 weeks produced at the Fukuchiyama Experimental Farm, located in Kyoto Prefecture, of Takeda Chemical Industries, were used. The 15 pigs were divided randomly into two groups: a control

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Table I. Weights and Feed Intake of Swine Treated with Sedecamycin

expt	treatment level, ppm	pig no.	body wt, kg		weekly feed intake/head, kg				total feed intake, kg	sedecamycin intake/head, mg		
			start	finish	1	2	3	4		total	per kg/day overall	per kg/day last week[a]
1	250	3	7.1	21.6	3.87	4.66	6.37	7.98	22.87	5718	14.84	14.77[b]
	250	9	20.3	39.4	8.50	9.42	10.97	10.87	39.76	9940	11.65	10.38[b]
	control	3	6.9	19.3	3.33	4.75	6.09	6.90	21.07			
2	50	15	27.3	38.2	14.75	16.41			31.16	1558	3.39	3.28
	500	15	26.7	38.2	13.06	15.53			28.59	14295	31.30	31.50
	control	3	26.3	37.9	13.83	15.40			29.23			

[a] Feed intake in the last week (kg) × treatment level (mg/kg)/(body weight at 7 days before final + final body weight) × $^2/_7$. [b] Weighed average of these two values, 11.15.

Table II. Recoveries of Sedecamycin and Its Metabolites Added to Control Blood and Tissue

tissue	added, ppm	recovery (mean ± SD), % I	II	III	IV	replicates
plasma	1.0	87.2 ± 0.5	94.2 ± 1.2	97.1 ± 5.6	99.6 ± 5.5	4
	0.2	91.2 ± 8.7	96.0 ± 2.8	95.2 ± 2.1	101.9 ± 9.0	4
	0.05	79.0 ± 2.5	91.7 ± 0.7	92.9 ± 0.7	95.0 ± 4.2	2
serum	0.5	74.3 ± 4.9	77.9 ± 4.1	90.6 ± 3.0	84.8 ± 3.0	3
liver	1.0	92.5 ± 1.3	92.0 ± 3.4	87.5 ± 3.8	81.3 ± 7.6	4
	0.5	94.0 ± 2.0	87.3 ± 14.2	85.7 ± 4.2	85.3 ± 2.5	3
	0.2	89.6 ± 7.2	81.4 ± 8.5	84.4 ± 7.0	78.4 ± 6.5	5
	0.05	85.0 ± 11.5	85.6 ± 5.1	75.3 ± 4.5	86.7 ± 11.2	3
kidney	1.0	87.2 ± 5.0	88.8 ± 3.0	85.5 ± 3.9	79.2 ± 5.5	4
	0.5	94.0 ± 1.0	93.0 ± 1.0	92.3 ± 6.4	86.0 ± 2.0	3
	0.2	88.2 ± 3.5	89.8 ± 3.0	80.2 ± 5.5	75.0 ± 8.3	4
	0.05	76.5 ± 16.3	84.0 ± 6.1	68.5 ± 12.0	82.0 ± 5.7	2
muscle	1.0	95.0 ± 1.7	91.4 ± 5.5	88.6 ± 5.2	74.4 ± 5.5	5
	0.5	87.7 ± 2.1	81.0 ± 3.0	78.0 ± 2.0	67.0 ± 4.4	3
	0.2	88.2 ± 1.5	85.0 ± 2.7	77.8 ± 3.2	71.8 ± 4.9	4
	0.05	84.0 ± 12.7	86.0 ± 2.8	69.0 ± 2.8	78.5 ± 13.4	2
fat	1.0	82.8 ± 3.9	80.2 ± 4.1	79.5 ± 5.1	73.2 ± 7.1	4
	0.5	90.7 ± 0.6	92.7 ± 2.3	85.0 ± 4.6	74.7 ± 4.0	3
	0.2	85.5 ± 4.8	80.5 ± 7.6	74.8 ± 4.0	71.5 ± 5.4	4
	0.05	82.3 ± 13.6	78.0 ± 8.2	75.7 ± 15.9	78.0 ± 4.4	3
ileum	0.5	93.0 ± 1.0	89.3 ± 2.5	67.0 ± 3.0	86.3 ± 7.8	3
colon	0.5	89.7 ± 1.5	88.0 ± 1.7	78.9 ± 0.2	90.0 ± 6.6	3

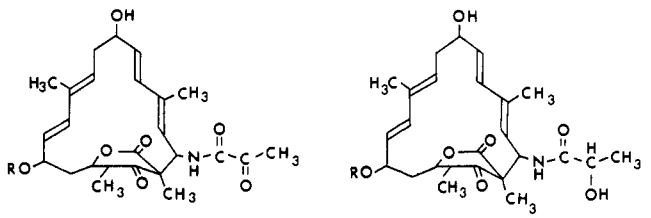

Figure 1. Structure of sedecamycin and its metabolites.

of three and a treated group of 12. The control animals were fed a control diet. The treated animals were fed a diet containing I at 250 ppm. The pigs were given a diet and water ad libitum for 28 days. The control animals and three of the treated animals were slaughtered 2 h after the feed was removed (0 day of withdrawal). The remainder were placed on the control ration. After 1, 3, and 7 day(s), three pigs each were slaughtered.

In experiment 2, 33 L × W × D pigs aged 10 weeks obtained from Chidoriya Chikusan Co., Ibaragi Prefecture, were used. They were fed a basal ration for 1-week acclimation. None of the pigs showed any clinical abnormality. They were randomly assigned to a control group of three pigs and two medication groups of 15 each.

The pigs were provided with a diet and water ad libitum. The animals in each of the two experimental groups were fed a diet containing I at 50 or 500 ppm, respectively, for 14 days. The control pigs were fed an unmedicated diet. The three control pigs and three pigs from each of the medicated groups were killed 2 h after the diet was removed. The remaining 12 animals in each treated group were fed the unmedicated diet. Three pigs in each treated group were slaughtered 1, 3, 5, or 7 day(s) after withdrawal of the drug.

Tissue Sampling. At designated withdrawal times, pigs were slaughtered by electric shock and exsanguination after blood samples were collected. Samples from the kidneys, liver, muscle (longissimus dorsi), and back fat were collected. Plasma (in experiment 1) and serum (in experiment 2) were separated from the blood. In addition to the tissue samples noted above, the small intestine (ileum) in experiment 1 and ileum and large intestine (colon) in experiment 2 were collected and washed thoroughly with water. All samples were frozen and stored at −20 °C until analysis.

Residue Analysis. *Extraction.* I–IV were extracted by the method developed by Okada and Kondo. The samples of solid tissues (liver, kidney, muscle, fat, ileum, colon) were cut into cubes smaller than 5 mm. The samples were selected so as to avoid particularly bloody or fatty areas (except for fat). A 10-g sample was placed in a 50-mL round-bottom centrifuge tube. Ten milliliters of 0.1 M phosphate buffer (pH 4.5) containing 10% NaCl (w/v) was added to the tube, and the tissue was homogenized with an Ultra-Turrax TP-18 homogenizer (Janke & Kunkel GmbH, West Germany) in an ice bath. The homogenate was extracted three times with 10-mL aliquots of ethyl acetate. Each time the mixture was stirred with the homogenizer at room temperature and centrifuged to separate the organic layer. The ethyl acetate extracts were combined in a 100-mL round-bottom flask and evaporated

Table III. Residue Concentration at Zero Withdrawal of Sedecamycin and Its Meabolites in Blood and Tissues of Swine Fed Sedecamycin

expt	treatment level, ppm	pig no.	tissue	tissue concentration, ppm I	II	III	IV
1	250	7	plasma	NDR[a]	NDR	NDR	NDR
			liver	NDR	NDR	NDR	tr[b]
			kidney	tr	tr	tr	NDR
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	0.048	0.058	0.039	0.141
		8	plasma	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	0.076
			kidney	tr	tr	tr	NDR
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	0.084	0.058	NDR	0.162
		9	plasma	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	tr
			kidney	0.028	NDR	tr	NDR
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	0.038	0.025	0.044	0.098
2	50	1	serum	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	NDR
			kidney	NDR	NDR	NDR	NDR
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	NDR	NDR	NDR	0.080
			colon	NDR	NDR	tr	NDR
		2	serum	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	tr
			kidney	NDR	NDR	NDR	NDR
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	NDR	NDR	NDR	NDR
			colon	NDR	NDR	tr	tr
		3	serum	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	NDR
			kidney	NDR	NDR	NDR	NDR
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	NDR	NDR	NDR	NDR
			colon	NDR	NDR	tr	NDR
2	500	16	serum	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	tr
			kidney	NDR	tr	NDR	tr
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	NDR	NDR	0.11	0.62
			colon	NDR	tr	0.13	0.12
		17	serum	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	0.05
			kidney	NDR	tr	NDR	tr
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	tr	NDR	NDR	tr
			colon	NDR	tr	0.11	0.08
		18	serum	NDR	NDR	NDR	NDR
			liver	NDR	NDR	NDR	0.06
			kidney	NDR	tr	NDR	tr
			muscle	NDR	NDR	NDR	NDR
			fat	NDR	NDR	NDR	NDR
			ileum	0.13	tr	0.16	0.63
			colon	NDR	NDR	0.04	0.05

[a] No detectable residue. [b] tr indicates the presence of residue less than the detection limits.

to dryness. The residue was reconstituted with 5 mL of dichloromethane by an ultrasonic generator and transferred to a silica gel–Florisil cleanup column (vide infra).

The plasma or serum was thawed, and 5 g was placed in a 50-mL round-bottom centrifuge tube. The sample was extracted three times with 10-mL portions of ethyl acetate at room temperature by stirring with an Ultra-Turrax. Each time, the layers were separated by centrifugation. The ethyl acetate extracts were combined and removed by rotary evaporation (40 °C). The residue was dissolved in 1 mL of ethyl acetate for HPLC determination.

Cleanup Column. The column was prepared by placing, in succession, 3 cm of silica gel, 1.5 cm of Florisil, and 2 cm of anhydrous Na_2SO_4 in dichloromethane in a glass column of 1.1-cm i.d. fitted with a coarse fritted disk and stopcock. The tissue extract was transferred to the column, and 75 mL of dichloromethane–ethyl acetate (90:10, v/v) was added. Sedecamycin and its metabolites were eluted from the column with a mixture of ethyl acetate–acetone (80:20, v/v). The first 20 mL of eluate and a subsequent

30 mL of eluate were collected separately. Half of the first eluate was evaporated to dryness and redissolved with 1 mL of ethyl acetate to determine I–III. The other half of the first eluate and half of the second were combined, evaporated to dryness, and dissolved with 1 mL of ethyl acetate to determine IV.

HPLC Analysis. A Model 6000A pump and U6K injector (Waters Associates, Milford, MA) with a SPD-2A ultraviolet detector (Shimadzu Corp., Kyoto, Japan) were used with a Waters μPorasil column (30 cm × 3.9 mm). The mobile phases were hexane–2-propanol (80:20, v/v) at a flow rate of 1.5 mL/min at 600–1000 psi for I–III and hexane–2-propanol–acetic acid (75:25:0.2, v/v) at a flow rate of 1.5 mL/min at 700–1000 psi for IV. The injection volumes were 20 μL for I–III and 40 μL for IV. The eluent was monitored at 227 nm. Peak heights were used for quantitation.

The presence of I in the kidney extract was confirmed by μBondapak C_{18} and acetonitrile–0.01 M phosphate buffer (pH 8.2) (40:60) at a flow rate of 1.0 mL/min at 700–1100 psi, when it was ambiguous with normal-phase HPLC.

RESULTS AND DISCUSSION

The weights and feed intake of pigs (per group) in each experiment are summarized in Table I. No significant differences were observed in weight gains and feed intake among the control, 50 ppm, and 500 ppm treated groups. A comparison between the control and the 250 ppm treated group is meaningless because of the big difference in starting age. Since the drug residue level in tissues is considered to be positively associated with the amount of drug administered immediately before slaughter, drug intake (per kilogram of body weight per day) during the last week of the medication period was compared with overall drug intake throughout the experiments. The two figures calculated were almost the same, indicating that the pigs had taken the same amount of feed per kilogram of body weight throughout the experiments. The drug-intake ratio in the last week (1:3.4:9.6) did not necessarily accord with the ratio of the drug content in feed (1:5:10); however, the drug intake, even in the 250 ppm group, greatly exceeded that of the maximum dose (75 ppm) to be used therapeutically in swine infection.

The analytical method was validated by the recovery of the compounds from control samples spiked at 0.05–1.0 ppm. The average recovery of known amounts of the compounds from control tissues ranged from 67% to 102% (Table II). Straight lines passing through zero point were obtained with peak heights for the standard solutions of each compound from 0.25 to 5 μg/mL (corresponding to 0.05–1.0 ppm in tissue). The detection limits, defined as 99% upper confidence limits of the intercept of the standard lines, were 0.012 ppm for I, 0.028 ppm for II, 0.026 ppm for III, and 0.044 ppm for IV (Okada and Kondo, 1987).

Tissue residue of the pigs at 0 day of withdrawal are shown in Table III. No residue of I–IV was detected in all samples of blood, muscle, and fat at all doses. Moreover, this study does not show any appreciable potential for I to accumulate in the adipose tissue even at the 500 ppm dose, in spite of concern that it might be deposited in adipose tissue because of its high lipophilicity.

At least 57% of I was known to be absorbed by swine when it was administered orally (Okada et al., 1984). Nevertheless, this study demonstrates that I, along with its metabolites, is quickly metabolized and disappears rapidly from the blood; consequently, they do not appear in muscle and fat.

The drug residue was observed more or less in the ileum, colon, liver, and kidney. The ileum showed the highest residue levels at all doses. At the 50 ppm treatment level, trace amounts of III and IV were detected in the colon and liver. At the 250 ppm treatment, trace levels of residue for I–III were found in kidney, while liver showed only little higher levels of IV than at 50 ppm. At the 500 ppm treatment, the colon showed the second highest residue levels. While levels of III and IV in the ileum were greater than those at the 250 ppm treatment, levels of I and II were somehow smaller than those at 250 ppm. Trace to low levels of drug residues were found in the liver and kidney. Although the residue levels of the drugs varied greatly among individuals and the compounds tested, it can be concluded that the drug residue roughly corresponded to the feed levels of I.

There are two possibilities for the highest drug residue found in the ileum: First, the metabolites excreted into the bile are reabsorbed in the ileum. Second, the metabolites are directly excreted across the intestinal mucosa into intestinal lumen (Dayton et al., 1983). No evidence is available for either of the possibilities. The presence of some residue in the liver and kidney can be explained by the fact that these organs are involved in the excretion of I and its metabolites (Okada et al., 1984).

At 1 day after withdrawal and thereafter, no compounds (I–IV) were detected in any tissue (data not shown), indicating that I and its metabolites are eliminated very rapidly from all tissues, and a 1-day withdrawal period can be proposed.

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