Milbemycin D Concentrations in Tissues after Oral Administration in Collies, Shelties and Japanese Mongrel Dogs

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ABSTRACT. Milbemycin D (Milbe) concentrations in plasma and tissues of the central nervous system and some other organs were determined 3 hr after oral administration in rough-coated collies (Collies), Shetland sheep dogs (Shelties) and Japanese mongrel dogs (Mongrels), and the relationships among the plasma and tissue Milbe concentrations and neurologic signs were investigated. No mongrels administered 5.0 mg/kg body weight of Milbe showed neurologic signs, but 3 of 4 Collies and 2 of 4 Shelties thus administered and 2 of 4 mongrel dogs administered 12.5 mg/kg of Milbe developed signs such as salivation, staggering and tremor. The Milbe concentrations in dogs with neurologic signs were significantly higher than in dogs without the signs in the plasma, cerebrum, cerebellum and medulla oblongata. However, there were no significant differences in tissue/plasma ratio of Milbe concentration between the dogs with and without neurologic signs except cerebrum/plasma ratio between the mongrel 12.5 mg/kg and Collie-Shelty groups. The plasma concentrations of Milbe were significantly correlated with the concentrations in the cerebrum, cerebellum, medulla oblongata, liver and kidney. It was considered that Milbe could penetrate the brain through the blood-brain barrier depending on the plasma concentration, and might cause the neurologic signs.—**KEY WORDS:** collie, milbemycin D, neurologic signs, shelty, tissue concentration.

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In separate reports [13, 12], the authors described the high sensitivity for milbemycin D (Milbe) in rough coated collies (Collies) and Shetland sheep dogs (Shelties) just as for ivermectin [8, 10]. The plasma levels of Milbe in dogs with neurologic signs were higher than those in dogs without the signs after oral administration of Milbe. In ivermectin, the high sensitivity in Collies was attributed to the singularity of the blood-brain barrier (high penetration of the drug). However, the data about plasma levels of Milbe suggested that the bioavailability of the compound might be greater in some Collies and Shelties (described in the separate paper). The high sensitivity in Collies and Shelties might not associate with the singularity of the blood-brain barrier. The data about Milbe concentrations in the various tissues, especially in the central

nervous systems (CNS), will provide more certain information about the high sensitivity for Milbe in Collies and Shelties. In the present study, the concentrations of Milbe in the plasma and tissues including the CNS were determined, and discussed the relationships between the concentrations of Milbe and development of neurologic signs.

MATERIALS AND METHODS

Eight mongrel dogs (mongrels), 4 Collies and 4 Shelties were used. A powder preparation containing 1% Milbe (Sankyo Co., Ltd., Tokyo) was administered orally to 4 mongrels, 4 Collies and 4 Shelties at a dose of 5.0 mg/kg body weight, and to 4 mongrels at a dose of 12.5 mg/kg body weight. Age, sex and body weight of the experimental dogs are shown in Table 1. Some dogs were

Table 1. Profiles of experimental dogs

Breed	Dose (mg/kg)	No. of dogs			Age (year)	Body weight (kg)
		Total	Male	Female		
Mongrel	5	4	1	3	1 - 4	6- 7
Mongrel	12.5	4	0	4	0.7- 4	5- 8
Collie	5	4	0	4	4 -10	10-17
Shelty	5	4	1	3	5 -10	5- 8

infected with heartworms. However, heartworm-infected dogs were used for the study after elimination of microfilariae with the oral administration of 1.0 mg/kg of Milbe, and no difference was found in Milbe concentrations between the dogs with and without heartworm infection, so the data were shown together. The experimental dogs were checked for clinical signs before and after administration. After collection of peripheral blood, the dogs were euthanized at 3 hr after administration by exsanguination after anesthesia. The liver, bile, kidney, cerebrum, cerebellum, medulla oblongata, spinal cord and cerebrospinal fluid were sampled from each dog. The concentration of Milbe was determined with a HPLC-fluorescence spectrophotometer system [5] as follows; Milbe was extracted from 1 g of each tissue into 5% ethyl acetate-hexane after homogenization. After the clean-up procedure with a silica column (Bond Elute-si, Analytical International, Harbor City, U.S.A.), Milbe was converted into dehydro-milbemycin D, a fluorescent derivative of the compound, with addition of triethyl amine and trifluoro acetic anhydride. After excretion and evaporation, the residue was resolved into 100% acetonitrile (mobil phase), and injected into high-performance liquid chromatography (655A-12 Liquid Chromatograph, Hitachi, Ltd., Tokyo). For separation, detection and data analysis, a Radial-PAK C₁₈ column (Waters Associates, Milford, U.S.A.), a fluorescence spectrophotometer (F-1000, Hitachi,

Ltd., Tokyo) and a data analyser (Chromatopac C-R3A, Shimadzu Corp., Kyoto) were used, respectively. For comparison of data, Student's *t*-test or Duncan's multiple range test was used.

RESULTS

In Table 2, Milbe levels in plasma and tissues are shown. In the mongrel dogs administered 5.0 mg/kg body weight, the plasma Milbe level was 256.0 ± 153.3 ng/ml. Milbe was detected with high concentrations in the liver, bile and kidney. In the central nervous system (CNS), Milbe was detected with a considerably large concentration in the cerebrum, cerebellum and medulla oblongata in all dogs, but with low concentrations in the spinal cord and cerebrospinal fluid. At a dose of 5.0 mg/kg, Milbe levels in plasma and each tissue of Collies and Shelties were higher than those of mongrels, but the differences were not statistically significant. Milbe levels in each tissue of mongrels administered 12.5 mg/kg were slightly higher than in mongrels administered 5.0 mg/kg. The tissue/plasma ratio, as shown in Table 3, overlapped in each group. A statistically significant difference was found only in the cerebrum/plasma ratio between the mongrel 12.5 mg/kg and Collie-Shelty groups.

In the present study, not all Collies and Shelties showed the neurologic signs. As shown in Table 4, no mongrels displayed the signs, but 3 of 4 Collies and 2 of 4 Shelties

Table 2. Tissue milbemycin D concentrations in the mongrel and Collie-Shelty groups

Tissue	Tissue milbemycin D concentration (ng/g)								
	Mongrel (5 mg/kg) ^{a)}			Mongrel (12.5 mg/kg)			Collie-Shelty (5 mg/kg)		
	n	Mean	SD ^{b)}	n	Mean	SD	n	Mean	SD
Plasma ^{c)}	4	256.0	153.3	4	713.9	624.9	8	677.2	412.9
Cerebrum	4	149.3	100.5	4	183.7	139.4	8	448.8	274.9
Cerebellum	4	140.6	95.3	4	258.8	238.3	8	436.7	250.7
Medulla oblongata	4	165.4	105.6	4	257.5	210.6	8	347.0	220.8
Spinal cord	4	35.2	23.2	4	39.6	40.5	8	77.6	73.2
Cerebrospinal fluid	4	2.6	3.6	4	0.6	1.2	8	0.2	0.6
Liver	4	996.1	344.3	4	1276.9	887.9	8	2549.9	1699.4
Bile	4	1589.2	1892.6	4	1411.8	1503.1	8	2474.0	3661.7
Kidney	4	434.4	210.1	4	1139.5	780.2	8	1276.2	898.4

a) Dose.

b) Standard deviation.

c) Plasma concentration indicated in ng/ml units.

Table 3. Tissue/plasma ratio of milbemycin D concentrations in the CNS of the mongrel and Collie-Shelty groups

Organ	Tissue/plasma ratio of milbemycin D					
	Mongrel 5 mg/kg ^{a)} (n=4)		Mongrel 12.5 mg/kg (n=4)		Collie-Shelty 5 mg/kg (n=8)	
	Mean	SD ^{b)}	Mean	SD	Mean	SD
Cerebrum	0.558 (0.436–0.705) ^{d)}	0.114	0.282 (0.169–0.427)	0.120	0.736 (0.371–1.407)	0.361 ^{c)}
Cerebellum	0.580 (0.343–0.873)	0.269	0.376 (0.206–0.722)	0.238	0.711 (0.397–1.345)	0.316
Medulla oblongata	0.767 (0.334–1.493)	0.539	0.398 (0.207–0.668)	0.195	0.565 (0.219–1.385)	0.374
Spinal cord	0.144 (0.045–0.250)	0.084	0.051 (0 –0.119)	0.050	0.112 (0 –0.292)	0.034

a) Dose.

b) Standard deviation.

c) Significantly different for the mongrel 12.5 mg/kg group.

d) Range.

did so at a dose of 5.0 mg/kg body weight till 3 hr after Milbe administration. At a dose of 12.5 mg/kg, 2 of 4 mongrel dogs showed the signs. Therefore, Milbe levels were recalculated in the groups with and without neurologic signs (Table 5). The plasma Milbe level in the group with neurologic signs was significantly higher than in the group with-

out the signs. Also in the cerebrum, cerebellum, medulla oblongata and spinal cord, Milbe levels were significantly higher in the group with the signs, but not cerebrospinal fluid. The dogs with a high Milbe concentration tended to show the more severe signs. In the liver and kidney, the Milbe levels were also higher in dogs with the signs with

the signs than in dogs without them. The Milbe level in the bile tended to be high in dogs with the signs, but was not statistically significant. There were no significant differences in tissue/plasma ratio of the Milbe concentration between the dogs with and without neurologic signs (Table 6).

Table 7 shows the relationships between the plasma and tissue Milbe concentrations. The plasma Milbe concentration was significantly correlated with the liver and kidney Milbe concentrations. The plasma concentration was significantly correlated also with the CNS tissues such as cerebrum, cerebellum and medulla oblongata, but not with the spinal cord and cerebrospinal fluid. The correlation coefficient was relatively high between the plasma Milbe concentration and concentrations in the cerebellum (Fig. 1) or medulla oblongata (Fig. 2).

DISCUSSION

Milbe, just as ivermectin [9], stimulates the release of γ -aminobutyric acid (GABA) from rat brain synaptosomes [4]. In mam-

mals, GABA (GABAergic nerve) was found to be distributed in many places in the CNS such as cerebellum, cerebrum (substantia nigra), mesencephalon, pons, medulla oblongata, hypothalamus and soon [7], and it acts as an inhibitory transmitter [6, 7]. In the present study, high concentrations of Milbe were detected in the cerebellum, cerebrum and medulla oblongata in dogs showing neurologic signs, and the dogs having higher Milbe concentrations tended

Table 4. Incidence of neurologic signs

Breed	n	No. of cases	
		With neurologic signs ^{a)}	Without neurologic signs
Mongrel (5 mg/kg) ^{b)}	4	0	4
Mongrel (12.5 mg/kg)	4	2	2
Collie (5 mg/kg)	4	3	1
Shelty (5 mg/kg)	4	2	2
Total	16	7	9

a) Salivation, staggering and tremor.

b) Dose.

Table 5. Tissue milbemycin D concentrations in groups with and without neurologic signs

Tissue	Tissue milbemycin D concentration (ng/g)				P< ^{b)}
	With signs (n=7)		Without signs (n=9)		
	Mean	SD ^{a)}	Mean	SD	
Plasma ^{c)}	998.2	357.2	258.4	94.6	0.01
Cerebrum	509.9	240.6	150.3	101.4	0.01
Cerebellum	539.4	187.0	146.1	94.3	0.001
Medulla oblongata	447.4	164.1	148.5	103.0	0.001
Spinal cord	98.7	66.6	27.6	25.2	0.05
Cerebrospinal fluid	0.6	1.0	1.1	2.6	NS ^{d)}
Liver	2950.5	1533.2	982.0	464.2	0.05
Bile	3097.1	3796.5	1124.0	1295.4	NS
Kidney	1717.2	744.2	498.3	220.1	0.01

a) Standard deviation.

b) Probability of significant difference between the levels with and without neurologic signs.

c) Plasma concentration indicated in ng/ml units.

d) Not significant.

Table 6. Tissue/plasma ratio of milbemycin D concentrations in the CNS of dogs with and without neurologic signs

Organ	Tissue/plasma ratio of milbemycin D				P< ^{a)}
	With signs (n=7)		Without signs (n=9)		
	Mean	SD ^{a)}	Mean	SD	
Cerebrum	0.571	0.308	0.581	0.350	NS ^{c)}
	(0.169–1.088) ^{d)}		(0.198–1.407)		
Cerebellum	0.602	0.250	0.589	0.356	NS
	(0.206–0.976)		(0.232–1.345)		
Medulla oblongata	0.487	0.204	0.641	0.487	NS
	(0.207–0.774)		(0.219–1.493)		
Spinal cord	0.110	0.066	0.112	0.102	NS
	(0 –0.119)		(0 –0.292)		

a) Probability of significant difference.

b) Standard deviation.

c) Not significant.

d) Range.

Table 7. Relationship between the plasma and tissue milbemycin D concentrations

Tissue	n	r ^{a)}	P< ^{b)}
Cerebrum	16	0.58	0.05
Cerebellum	16	0.70	0.01
Medulla oblongata	16	0.68	0.01
Spinal cord	16	0.49	NS
Cerebrospinal fluid	16	–0.04	NS
Liver	16	0.64	0.01
Bile	16	0.39	NS ^{c)}
Kidney	16	0.59	0.05

a) Correlation coefficient.

b) Probability of significant correlation coefficient.

c) Not significant.

to show more severe signs. The neurologic signs observed after Milbe administration were not inhibited by administration of edrophonium chloride (unpublished data). Therefore, it was considered that the neurologic signs observed after Milbe administration were directly or indirectly associated with the pharmacological action of Milbe for the CNS.

Collies were more susceptible to the toxic effects of ivermectin than other breed dogs

[8, 10]. Ivermectin was highly detected in the CNS of Collies affected by the drug, and breed idiosyncrasy of Collies was attributed to the greater penetration of ivermectin through the blood-brain barrier [10]. In the present study, however, Milbe was detected in the CNS tissues in all mongrel dogs, Collies and Shelties administered 5.0 mg/kg body weight. The mongrel dogs administered 12.5 mg/kg, which also showed neurologic signs, had higher plasma and CNS concentrations of Milbe just as in the Collies and Shelties showing neurologic signs. The tissue/plasma concentration ratio did not indicate the high penetration of Milbe into the CNS in dogs with neurologic signs, because the ratio was overlapped, and no statistically significant difference was found between the dogs with and without the signs. Moreover, the Milbe concentrations in the CNS tissues were significantly correlated with the plasma Milbe concentration. Generally, highly lipid-soluble drugs, such as chloramphenicol and diazepam, enter the brain readily [1]. Milbe, the same as other macrolide antibiotics [11], is easily soluble in common organic solvents, *n*-hexane, ben-

Fig. 1. Correlation between the plasma and cerebellum milbemycin D concentration.

- : Mongrel without signs
- : Mongrel with signs
- : Collie or Sheltie without signs
- : Collie or Sheltie with signs

Fig. 2. Correlation between the plasma and Medulla oblongata milbemycin D concentrations.

- : Mongrel without signs
- : Mongrel with signs
- : Collie or Sheltie without signs
- : Collie or Sheltie with signs

zene, ethanol, methanol, and chloroform, and little soluble in water [13]. From these facts and data in the present study, It was considered that Milbe could enter the CNS tissues through the blood-brain barrier depending upon the plasma level of Milbe.

The difference in susceptibility to the

toxic effect of Milbe between the mongrel dogs and dogs of Collie and Sheltie breed was dependent on the difference in the plasma concentration of Milbe, but there was no difference in drug penetration through the blood-brain barrier. Plasma concentration of the drug is dependent on

absorption from the intestine and metabolism and excretion in the liver [2]. It is appropriate to consider that the reason for the difference in the plasma Milbe concentration between the mongrel dogs and Collies of Shelties may be latent in a difference mainly in the bioavailability of the drug.

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要 約

コリー、シェルトイ及び雑種犬に経口投薬されたミルベマイシン D の組織内濃度：佐々木栄英・北川 均・石原勝也・石塚清美（岐阜大学農学部家畜内科学講座）——コリー、シェルトイ及び雑種犬にミルベマイシン D（ミルベ）を経口投与し、血漿及び組織の濃度を測定するとともに、血漿及び組織のミルベ濃度と神経症状との関係を検討した。体重 1 kg あたり 5 mg を経口投与した雑種犬では、1 例も神経症状を発症しなかった。しかし、コリーでは 4 例中の 3 例で、シェルトイでは 4 例中 2 例で流涎、歩様蹠踉及びび振せん等の神経症状を発症した。また、雑種犬でも 12.5mg/kg の投与で 4 例中 2 例が神経症状を発症した。投与 3 時間後、コリー、シェルトイ及び雑種犬の全例において、ミルベは血漿、大脳、小脳、延髄、肝臓、胆汁及び腎臓で検出され、それらのミルベ濃度は神経症状発症例において非発症例より有意に高値であった。血漿ミルベ濃度は、大脳、小脳、延髄、肝臓及び腎臓のミルベ濃度と有意に相関し、神経症状の発症は高い血漿ミルベ濃度と関連するように考察された。Ivermectin で示唆されているような血液脳関門の特殊性は、ミルベについてはコリー及びシェルトイでも認められなかった。