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Clinical Application of Milbemycin D as a Prophylactic Agent against *Dirofilaria immitis* Infection in Dogs: Sensitivity for the Drug in Rough-Coated Collies

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ABSTRACT. To evaluate sensitivity owing to breed idiosyncrasy for milbemycin D (Milbe), 5 rough-coated collies and 7 Shiba dogs were treated with Milbe at a dose of 1.0 mg/kg, 2.5 mg/kg and 5.0 mg/kg orally for 10 consecutive days. In the 5.0 mg/kg dose of the collie group, neurologic signs such as arrhythmia, salivation, mydriasis, staggering, recumbency, lethargy and so on occurred in 4 of 5 dogs after the 1st administration, and showed intermittently or continuously during the trial. The intensity of the neurologic signs was different in the individual dog and with the day of administration. The affected dogs recovered without treatment within 24 hr after the administration and no dog died.—**KEY WORDS:** breed idiosyncrasy, collie, milbemycin D.

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Milbemycin D (Milbe) is a macrolide derivative with a broad spectral anti-parasitic efficacy [12]. In dogs, the minimal effective oral prophylactic dose against *Dirofilaria immitis* (heartworm) infection was 1 mg/kg body weight [10, 11]. The oral administration of Milbe up to 5.0 mg/kg showed no clinically significant adverse reactions in circulating microfilaria negative dogs [8, 9]. Ivermectin is also a macrolide derivative with a broad spectral anti-parasitic efficacy as well as heartworm larvae. Collie are more sensitive than other breeds to toxic effects of ivermectin [1, 7], and it has been suggested that breed idiosyncrasy may be involved [7]. Therefore, it is expected that the collie may also have a high sensitivity for Milbe, but it has not been researched. The purpose of this study is to evaluate sensitivity owing to breed idiosyncrasy for Milbe in collie.

Five rough-coated collies, healthy and heartworm-free, consisting of 2 females and

3 males and aged from 3 to 5 months old at the start of the first trial, were used (collie group). Seven or five Shiba dogs of almost the same ages were used as the controls (Shiba dog group). These dogs were not consanguineous. Milbe (Sankyo Co., Ltd. Tokyo) was used as a powder preparation containing 1%. Firstly, Milbe in gelatin capsules was administered orally to the fasting dogs at a dose of 1.0 mg/kg of body weight once a day for 10 consecutive days (1.0 mg/kg trial). After a 2-week withdrawal period, 2.5 mg/kg was administered to the dogs once a day for 10 days (2.5 mg/kg trial). Moreover, after a 2-week withdrawal period, 5.0 mg/kg was administered to the dogs once a day for 10 days (5.0 mg/kg trial). The behavior and clinical signs of dogs were carefully observed until 6 hr after administration. Clinical examination and blood sampling were made before the first administration and 24 hr after the 1st administration in all dogs, and at the time

abnormal findings were found.

Only slight arrhythmia was observed in the 1.0 mg/kg and 2.5 mg/kg trials of both the Shiba dog and collie groups, and in the 5.0 mg/kg of the Shiba dog group on clinical signs. Arrhythmia, observed before administration in 1 or 2 dogs in every group, continued intermittently or continuously throughout the experiment at almost the same intensity. Slight arrhythmia appeared newly from the 1st or 5th administration in the 1.0 mg/kg and 2.5 mg/kg trials of the Shiba dog and collie groups. The collie group evidenced a higher incidence of arrhythmia after administration than the Shiba dog group (Table 1). In the 5.0 mg/kg trials of the collie group, moderate or severe sinus arrhythmia appeared in all dogs from the 1st or 2nd administration, and was observed on almost every day during the

experiment. Four of them displayed some neurologic signs from 70 to 180 min after administration. One dog (No. 850) showed the signs only after the 1st administration, the other 3 cases showed them intermittently or continuously during the experiment (Table 2). The intensity of the signs was different in the individual dog and on different days of administration. The slight cases showed inactivity, posterior-limb ataxia, staggering, excessive salivation, rough vesicular breath sound, and active peristaltic sound. The moderate cases presented hypersensitivity to the sound, ventral recumbency, mydriasis or semicoma in addition to the signs of slight case. In the severe cases, lethargy or tremor occurred after the slight or moderate signs. The moderate and severe signs kept on for 1 or 3 hr, then disappeared gradually, and recovered to almost normal state at 24 hr post-administration without treatment, and no dog died.

The clinical data in collies displaying the neurologic signs after the 1st administration in the 5.0 mg/kg trial are shown in Table 3. Blood pressure, RBC count, serum total protein concentration and serum osmolality decreased at the onset time of the neurologic signs. These findings suggested cardiac dysfunction, hemodilution or dilation of arterioles, and might be a result of auto-

Table 1. Number of dogs with arrhythmia before and after administration of milbemycin D

Trial	Time of arrhythmia appearance			
	Collie		Shiba dog	
	Before	After	Before	After
1.0 mg/kg	1/5 ^{a)}	4/4	1/7	1/6
2.5 mg/kg	2/5	2/3	1/7	1/6
5.0 mg/kg	0/5	5/5	2/5	0/3

a) Number of dogs with arrhythmia/number of dogs examined.

Table 2. Neurologic signs^{a)} in the collie group of the 5.0 mg/kg trial

Dog No.	Day of administration									
	1	2	3	4	5	6	7	8	9	10
847	++	—	+	—	—	++	+	—	—	—
848	+++	+	+	++	+	—	—	+	—	—
849	+	+	++	+	++	++	+++	+	+	+
850	+	—	—	—	—	—	—	—	—	—
865	—	—	—	—	—	—	—	—	—	—

a) —: No signs, +: Slight (Staggering and salivation), ++: Moderate (Mydriasis, ventral recumbency and semicoma in addition to the signs of slight cases), +++: Severe (Lethargy and tremor in addition to the signs of slight and moderate cases).

Table 3. Clinical data in collies developing neurologic signs of the 5.0 mg/kg trial

Item	No. of dogs	Before	1.2-3 hr ^{a)} after	6 hr after	24 hr after
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Body temperature (°C)	4	39.6 ± 0.6	39.0 ± 0.6	38.5 ± 0.2	38.5 ± 0.2
Heart rate (/min)	4	144 ± 7.2	130 ± 13.4	138 ± 12.8	106 ± 16.1
Respiratory rate (/min)	3	64 ± 11.3	37 ± 1.4	38 ± 11.3	23 ± 1.4
Systolic blood pressure (mmHg)	4	115 ± 8	91 ± 8	103 ± 20	114 ± 12
RBC ($\times 10^4/\mu l$)	4	544 ± 23	487 ± 23	496 ± 37	490 ± 24
WBC ($\times 10^2/\mu l$)	4	96.5 ± 7.0	105.3 ± 17.0	128.8 ± 12.3	88 ± 14.9
Total protein (g/dl)	4	6.0 ± 0.5	5.5 ± 0.4	5.7 ± 0.3	5.8 ± 0.3
A/G ratio	4	0.96 ± 0.06	0.88 ± 0.22	0.96 ± 0.14	0.88 ± 0.03
Osmolality (mOsm/kg)	4	286 ± 6	277 ± 8	276 ± 4	276 ± 2
GOT (IU/l)	4	35.5 ± 7.7	40.5 ± 9.9	38.8 ± 11.2	32.2 ± 4.2
GPT (IU/l)	4	27.3 ± 4.6	28.8 ± 6.0	27.2 ± 3.8	26.4 ± 8.7
ALP (IU/l)	4	260 ± 109	268 ± 124	242 ± 94	252 ± 87

a) At the time of neurologic sign onset.

onomic nervous system disturbance to control heart and vessels. Body temperature, heart rate and respiratory rate decreased gradually after administration. WBC count increased by only 6 hr. There were no significant changes in serum enzyme activities. There are no significant differences in clinical data between before the first administration and 24 hr after the 1st administration in 3 trials in both the Shiba dog and collie groups. There were no significant findings on autopsy.

It was considered that the neurological signs observed after Milbe administration might be effects of disturbance of the autonomic nervous system and central nervous system (CNS). Though pharmacological actions of Milbe have not been clarified sufficiently, Milbe stimulates the release of γ -aminobutyric acid (GABA) from rat brain synaptosomes [3]. Though the detailed action of GABA was not determined clearly in living mammals, it has been considered that the GABAergic nerve acted as an inhibitory nerve in CNS [2, 4, 5]. The neurologic signs of the collies might suggest the effects of Milbe on the GABAergic nerve.

Pulliam *et al.* [7] considered that the

higher concentration of ivermectin in CNS of Collie developing neurologic signs indicated a greater penetration of ivermectin through the blood-brain barrier. On the other hand, authors found a higher blood concentration of Milbe in collie rather than in mongrel dogs from 3 hr to 6 hr after Milbe administration of 5.0 mg/kg (unpublished data). This evidence, they indicated, may be related to the higher sensitivity of the collie for Milbe. These data will be reported elsewhere.

The results of the present study showed rough-coated collie had a higher sensitivity for Milbe. However, there were few problems in use of Milbe as a prophylactic agent against heartworm infection in collie. Because, there were no clinical signs except slight arrhythmia at a recommended dose of 1 mg/kg.

REFERENCES

1. Campbell, W. C., and Benz, G. W. 1984. *J. Vet. Pharmacol. Therap.* 7: 1-16.
2. Enna, S. J., and Gallagher, J. P. 1983. *Int. Rev. Neurobiol.* 24: 181-212.
3. Kozuka, M., Kunieda, Y., and Iwata, N. Unpublished data.

4. Obata, K. 1972. *Int. Rev. Neurobiol.* 15: 167-187.
5. Okada, Y. 1978. *Metabol. Dis.* 15: 327-340 (in Japanese).
6. Pong, S. -S., Wang, C. C., and Fritz, L. C. 1980. *J. Neurochem.* 34: 351-358.
7. Pulliam, J. D., Seward, R. L., Henry, R. T., and Steinberg, S. A. 1985. *Vet. Med.* 80: 33-40.
8. Sakamoto, T., Seki, I., Kikuchi, K., Nakahara, H., and Ogasawara, H. 1984. *J. Fac. Agr. Iwate Univ.* 17: 197-209.
9. Sasaki, Y., Kitagawa, H., Okachi, H., Kajita, Y., and Ishihara, K. 1986. *Jpn. J. Vet. Sci.* 48: 579-586.
10. Shiramizu, K., and Abu, M. 1985. *J. Jpn. Vet. Med. Assoc.* 38: 353-356 (in Japanese).
11. Tagawa, M., Takiyama, A., Ejima, H., and Kurokawa, K. 1985. *Jpn. J. Vet. Sci.* 47: 787-790.
12. Takiguchi, Y., Mishima, H., Okuda, M., and Terao, M. 1980. *J. Antibiotics* 33: 1120-1127.

要 約

犬糸状虫予防剤ミルベマイシンDの臨床応用：ラフコリーにおける薬剤感受性：佐々木栄英・北川 均・梶田祐司・岡地啓之・石原勝也（岐阜大学農学部家畜内科学講座）——5頭のラフコリーと7頭の柴犬にミルベマイシンDの1.0 mg/kg, 2.5 mg/kg および5.0 mg/kg を、それぞれ2週間の休薬期間をおいて1日1回10日間経口投与し、犬種による薬剤感受性の差を検討した。5.0mg/kgのコリー群では、第1回投薬後から5頭中4頭に不整脈、流涎、散瞳、歩様蹣跚、伏臥および嗜眠などの神経症状が間欠的または連続的に認められた。症状の程度は個体および投薬日により異なっていたが、投薬後24時間以内に回復し、死亡例はなかった。