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Prevention of *Toxoplasma* Oocyst Excretion by Cat with 2-sulfamoyl-4, 4′-diaminodiphenylsulfone (SDDS)

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Although the role of the cat in the natural transmission of toxoplasmosis has not been explained in full, it is obvious that *Toxoplasma* oocysts excreted by the cat are transmitted by direct contamination with the cat feces or indirectly through various mediators such as fly (Wallace, 1971), cockroach (Wallace, 1972), earthworm (Dubey *et al.*, 1970), or contaminated soil (Ruiz *et al.*, 1973). In view of the successful example of prophylaxis in chicken coccidiosis, we considered that continuous feeding of a prophylactic might prevent oocyst excretion in cats which had been ingested *Toxoplasma* cysts, trophozoites or oocysts.

We now have succeeded in preventing oocyst excretion by the cats which had been fed *Toxoplasma* cysts of the Beverley strain by continuous medication in feed of 2-sulfamoyl-4,4′-diaminodiphenylsulfone (SDDS), the drug which is used as a therapeutic of swine toxoplasmosis (Shimizu *et al.*, 1968; Ohshima *et al.*, 1969) and also effective in preventing *Toxoplasma* infection in swine (Ohshima *et al.*, 1970, 1971; Shimizu *et al.*, 1970).

Materials and Methods

Eighteen young cats negative with dye- and hemagglutination (HA) tests for toxoplasmosis were used. They were housed individually in a steel cage and fed commercial canned catfood. *Toxoplasma* cysts of the Beverley strain in a saline emulsion of the brain of mice infected 1 month before the experiment were mixed in the morning meal on day 0 and given to the cats. The number of cysts administered was counted microscopically. Six out of the 18 cats were given 10% powder of SDDS in lactose once daily mixed in the evening meal at daily doses of 10 (4 cats) and 3 (2 cats) mg SDDS/kg. Other 12 cats were non-medicated control group. The medication was started 5 days before feeding the cysts and continued for 25 days.

Cat feces were collected daily. Processing of the feces was performed after the method of Dubey *et al.* (1970). Five grams of the feces were suspended in 10 volumes of tap water, and centrifuged at 1,000 rpm for 10 min and the supernatant was discarded. The sediment was resuspended in 35% sucrose solution containing 0.8% phenol. After centrifugation at 1,000 rpm for 10 min, 2 ml of the supernatant was aspirated from the surface. One to 2 drops of the supernatant were examined for presence of *Toxoplasma* oocysts microscopically and the remainder was diluted in 10 volumes of tap water and recentrifuged. The sediment was resuspended in 2.5% potassium dichromate solution, poured into a plastic Petri dish to a depth of about 5 mm, and kept for more than 1 week at room temperature. The preserved fecal sample was washed and resuspended in 2 ml of tap water and 0.5 ml each of the suspension was then adiministe-

red orally into 2-3 mice by the stomach tube. The positive dye test titer of the serum and/or presence of cysts under microscopy in a portion of the brain of the mice 4 weeks later were the criteria for the existence of the oocysts in the cat feces.

The anti-toxoplasmic antibodies in cat serum were estimated by the dye- and HA tests 15 days and 1 month after feeding the cysts. The method of the dye test modified by Kobayashi *et al.* (1968) was employed and the HA test was performed using TOXO-TEST (Hiraoka and Ohshima, 1972).

Twelve cats (8 of control, 2 of 10 mg SDDS/kg/day group, and 2 of 3 mg SDDS/kg/day group) were sacrificed 1 month after the feeding of cysts and isolation of the living parasite was attempted from the brain, liver, lung and muscle by intraperitoneal inoculation of their homogenates into mice. The remainder of the cats were again fed cysts of the Beverley strain 45 days after the initial feeding and the feces were examined daily for 2 weeks.

Results

Results are shown in Table 1. *Toxoplasma* oocysts were excreted in feces of all the 12 control cats and 1 out of the 2 cats given 3 mg SDDS/kg/kay. The period of oocyst excretion varied, but it was longer in cats which were given more cysts as a general rule. Four cats of the group of SDDS 10 mg/kg/day and 1 of 3 mg/kg/day did not excrete the oocyst at all. Antibody titers as estimated by the dye- and HA tests did not rise in cats of the medicated groups and no living parasites were detected from their

Table 1 Prevention by SDDS of oocyst excretion in cats given *Toxoplasma* cysts of the Beverley strain

Dose of SDDS (mg/kg/day)	Cat No.	Body Wt. (g)	No. of Cysts Fed	Oocyst Excretion (Period*)	HA Test Titer		Dye Test Titer		*Toxoplasma* Isolation			
					Initial	After 1 Month	Initial	After 1 Month	Muscle	Liver	Brain	Lung
10	1—2	730	8400	—	1 : 32—1 : 32	<1 : 4-<1 : 4			—	—	—	—
	1—8	740	8400	—	<1 : 32—1 : 32	<1 : 4-<1 : 4			—	—	—	—
	2—1**	1320	24000	—	<1 : 32—1 : 32	<1 : 4-<1 : 4			ND***	ND	ND	ND
	2—4**	825	2400	—	<1 : 32—1 : 32	<1 : 4-<1 : 4			—	—	—	—
3	1—1	720	8400	—	<1 : 32—1 : 32	<1 : 4-<1 : 4			—	—	—	—
	1—6	620	8400	+(4—8)	<1 : 32—1 : 32	<1 : 4-<1 : 4			—	—	—	—
0(Control)	0—1	530	21760	+(3—13)	1 : 32-1 : 8192		ND		+	—	+	ND
	0—2	490	21760	+(3—16)	1 : 32-1 : 8192		ND		+	+	+	ND
	0—3	510	21760	+(3—13)	1 : 32-1 : 512		ND		+	+	+	ND
	1—3	1310	8400	+(4—5)	<1 : 32-1 : 256		<1 : 4-1 : 64		+	+	+	+
	1—4	680	8400	+(6—8)	<1 : 32-1 : 256		<1 : 4-1 : 256		+	—	+	+
	1—5	750	8400	+(7—8)	<1 : 32-1 : 64		<1 : 4-1 : 64		+	+	+	+
	1—7	810	8400	+(4—9)	<1 : 32-<1 : 32		<1 : 4-1 : 16		—	—	—	—
	1—9	660	8400	+(5—6)	<1 : 32-<1 : 32		<1 : 4-1 : 4		—	—	—	—
	2—2**	615	24000	+(4—10)	<1 : 32-<1 : 32		<1 : 4-<1 : 4		—	—	—	—
	2—3**	735	24000	+(5—22)	<1 : 32-<1 : 32		<1 : 4-<1 : 4		ND	ND	ND	ND
	2—5**	750	2400	+(5—8)	<1 : 32-<1 : 32		<1 : 4-<1 : 4		ND	ND	ND	ND
	2—6**	570	2400	+(4—10)	<1 : 32-<1 : 32		<1 : 4-<1 : 4		ND	ND	ND	ND

* The day after the feeding of cysts (the day of feeding: 0).

** These cats were again fed the cysts (47000/cat) 45 days after the initial feeding and did not show excretion of oocysts and antibody formation.

*** Not detected.

organs. In 6 control cats elevations of the antibody titers were observed and living parasites were isolated, but in the other 6 cats the rise of the antibody titers and isolation of the parasite were both negative in spite of the excretion of oocysts. Except in 3 control cats (Nos. 0-1, 0-2 and 0-2) which showed anorexia throughout the period of oocyst excretion no clinical symptoms appeared.

Two cats (Nos. 2-1 and 2-4) of the 10 mg SDDS/kg/day group and 4 of the control group (Nos. 2-2, 2-3, 2-5 and 2-6) were again fed 47,000 cysts of the Beverley strain per cat 45 days after the initial feeding. No oocyst appeared in their feces within 2 weeks and no rise of antibody titers was observed at 30 days after the refeeding.

Discussion

It is obvious that the continuous medication with SDDS mixed in daily food at a dosage of 10 mg/kg/day prevented cats from excreting oocysts after the feeding of the cysts. This conclusions is based on the evidence that oocyst excretion, elevation of antibody titers and isolation of the parasite were all negative in cats of the medicated group.

Six out of the 12 control cats excreted oocysts and showed the rise of antibody titers and the existence of living parasites in their organs, but in the other 6 control cats and in 1 medicated (3 mg SDDS/kg/day) cat the rise of antibody titers and the isolation of parasites were both negative ins pite of the excretion of oocysts. This finding is in accord with the observation by Dubey and Frenkel (1972) and it is considered, as these workers suggest, that in these cats *Toxoplasma* invasion may be confined only to the intestinal wall and the parasite may be eliminated after the oocyst production has ceased. These and 2 cats of the 10 mg SDDS/kg/day group neither excreted oocysts nor showed antibody formation after refeeding the cysts. Apparent immunity in the intestine may be established in these cats.

Murosaku (1973) has conducted a trial for prevention of pet cats from *Toxoplasma* infection as estimated by the HA test. Thirteen HA positive (1 : 256 or more) and 12 HA nagative cats were administered SDDS once a day in feed at the dosage of 10 mg/kg/day for 6 months. The HA negative cats remained negative throughout the period, and 7 of HA positive cats showed unchanged titers and 6 showed reduced titers after the 6 months. In this period among 6 untreated cats 2 out 4 HA negative cats became positive and 1 out of 2 HA positive cats showed a rise of titer. From these results Murosaku concluded that SDDS may be effective in preventing cats from *Toxoplasma* infection.

SDDS is known to be a safe drug (Sakuma *et al.*, 1968). We administered the drug to kittens for 3 months at dosages of 10, 20 and 40 mg/kg/day and examined their growth, blood cell count, blood picture, hematocrit, serum enzymes, serum protein etc. at monthly intervals, but no adverse effect of the drug was found (unpublished data).

We consider that the administration of SDDS to cats at the dosage of 10 mg/kg/day is a reliable method for prevention of the *Toxoplasma* infection and of the oocyst excretion which is thought to have a very important role on the infection of men and domestic animals.

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2-sulfamoyl-4, 4′-diaminodiphenylsulfone (SDDS) による猫のトキソプラズマ・オーシスト排泄予防

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トキソプラズマの自然感染における猫の役割は必ずしも明らかでないが，猫の排泄するトキソプラズマ・オーシストが，直接あるいは間接に，感染源となつていることは疑いない．著者らは，鶏コクシジウム症の予防と同じ考えで，予防剤を連用することにより，猫がトキソプラズマ（シスト，増殖型あるいはオーシスト）を摂取しても，オーシスト排泄を予防出来ると考え，実験を行なつた．

18頭のトキソプラズマ抗体陰性の幼猫を，1頭ずつケージに収容し，それぞれ Beverley 株シストを含むマウス脳乳剤を食べさせた．18頭中6頭には，2-sulfamoyl-4, 4′-diaminodiphenylsulfone (SDDS) を10 (4頭) および3 (2頭) mg/kg/day ずつ，シスト投与5日前から25日間，1日1回，餌に混じて与えた．シストを与えた後，毎日，糞中のトキソプラズマ・オーシストの有無を調べ，血清中抗体価を色素試験ならびに赤血球凝集試験によつて測定し，約半数の猫はシスト投与1カ月後に殺処分して，脳，肝，肺，筋肉からトキソプラズマ分離を試みた，残りの猫には，初回シスト投与から45日後に，再投与を行ない，経過を観察した．

実験成績は，以下の如くであつた．

1) 対照群12頭の全部と，SDDS 3 mg/kg/day 群の2頭中1頭は，トキソプラズマ・オーシストを排泄したが，SDDS 10 mg/kg/day 群の4頭全部と，3 mg/kg/day 群の1頭は，全く排泄しなかつた．

2) SDDS を投与した6頭では，血清中抗体価の上昇は認められず，トキソプラズマ分離も陰性であつた．

3) 対照群においては，12頭6頭が抗体価の上昇を示し，トキソプラズマ分離も陽性であつたが，残りの6頭は抗体価の上昇を認めず，内半数についてトキソプラズマ分離を行なつても陰性であつた．

4) オーシストを排泄したにもかかわらず抗体価の上昇が認められなかつた対照群6頭中4頭と，オーシストを排泄しなかつた SDDS 10 mg/kg/day 群の2頭は，シストを再投与したが，オーシストの再排泄は認められなかつた．

以上の結果から，SDDS を毎日 10 mg/kg/day ずつ猫に投与すれば，猫のトキソプラズマ感染あるいはオーシスト排泄を予防しうると考えられる．