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Toxoplasma gondii: Production of Acetylspiramycin-Resistance Strain, with Special Reference to Stability of its Resistance

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Verious species of bacteria have been reported to develop the resistance to many kinds of drug. In regard to protozoa, so far only the resistance of malaria parasites to anti-malarial drugs has been reported in different areas of the world by many investigators. However, no report on the development of complete, not partial, drug-resistant strain of *Toxoplasma gondii* has appeard, probably because of lack of effective drug on toxoplasmosis, especially on cyst-form of the organism in host animals.

The present paper deals with the development of *Toxoplasma* resistant to acetylspiramycin (Asp), one of macrolide antibiotics, by successive passages of the wild-type parasites through mice receiving treatment by the drug in the lower dosages. The resistance strain produced was examined for the stability of resistance by transferring the parasites successively to mice which were not given Asp. Virulence of resistance strain and cross resistance to sulfa drug were also examined.

Materials and Methods

Animals

The animals used for the experiments were ICR mice of both sexes weighing 20±1 gm. These mice have been raised in our laboratory and maintained in stainless-steel cages being separated by sex. M.F. Oriental Laboratory Chow and water were supplied *ad libitum*.

Parasites

RH strain of *T. gondii* was used throughout the present study. The strain has been maintained in our laboratory by intraperitoneal subinoculations into mice. This strain is highly virulent to mice, as it is well known.

Passages of parasites through Asp-treated mice

Approximately 3×10^4 RH-toxoplasmas were inoculated intraperitoneally into 12 mice. Half of the mice was given Asp immediately after inoculation with the parasites, and another half did not receive the drug at all. After recording the number of survived mice of both groups on day 6 of infection, they were sacrificed. Immediately after the sacrifice the number of parasites in the abdominal cavity was counted as follows; two ml of saline were injected into the peritoneal cavity and peritoneal fluid diluted by saline was taken after light massage of the abdomen. Number of parasites in the fluid was counted by means of hemocytometer. When the parasites wes too large in number, five fold dilution of the fluid was made by saline. The following passages were made by inoculating the parasites, which were collected from an Asp-treated mouse having maximum population in the peritoneal cavity, into clean mice.

Drug administration

Because Asp is soluble only in acid medium, distilled water added with 1/10 N H_2SO_4 in 24% was used as a solvent. However, as the dosage used in the present study was too large to dissolve Asp completely, the drug remaining unsolved in the solvent was suspended by shaking. For treatment, 0.5 ml of the suspension containing 8 mg or 16 mg (approximately 400 mg/kg or 800mg/kg) of Asp was given daily for 6 consecutive days by a stomack tube.

Estimation of the resistance of Toxoplasma to Asp and examination for the characteristics of Asp-resistant parasites

To estimate the development of resistance to Asp, 3,000 parasites obtained from the 18 th to 30 th passages through mice receiving the treatment with Asp were inoculated intraperitoneally into mice. The mice thus inoculated were devided into two groups; one group of mice were given 8 mg per mouse daily, while another group of mice received no Asp. The mean survival time of both groups of mice was compared each other and the resistance of the parasites to Asp was estimated. When the survival period of the infected mice and the rate of multiplication of the parasites in the peritoneal cavity of the mice were not affected by the treatment of the mice with Asp, the parasites were regarded as Asp-resistant, and these criteria were used for the determination of Asp-resistance. The daily dose of Asp given to mice was increased to 16 mg during 36 th and 100 th passage.

Stability of Asp-resistance of the parasites harvested from Asp-treated mice at 22 nd, 40 th, 50 th, 60 th and 100 th passages were examined by successive passages of the organisms through mice without adminstration of the drug. The organisms were collected from the mice at every 5 passages, and 3,000 parasites were inoculated into two groups of mice, consisting of 8 mice each. The one group of mice received 8 or 16 mg of Asp until death of mice, while another group of mice received no drug and mean survival time of both groups was compared.

Virulence of the Asp-resistance strain was compared with that of the Asp-sensitive wild-type RH strain. Inoculation with 1,000 parasites of Asp-resistant strains were obtained from Asp treated mice during 51 st and 100 th passage and the same number of wild-type RH strain made into the peritoneal cavity of two groups of mice. Mice thus inoculated were observed for the mean survival period.

In addition to the inoculation with resistant organism in large number (1,000) as described above, one organism of the resistance strain was inoculated into mice in order to check the resistance of the clone to the drug. The inoculum was isolated from Asp-treated mice at 55 th, 60 th, 80 th and 100 th passage using technique as follow: One drop of parasite suspension each was placed on a number of small cut pieces of sterilized cover slip. The number of parasite placed on the piece of cover slip was counted and the piece of cover slip containing only one parasite was inserted into the abdominal cavity of mouse by opening the abdominal wall. Multiplication of the organism in the peritoneal cavity of the mouse was examined by the same method as described above.

Asp-resistance strains developed were examined for the cross resistance to sulfa drug. The organism was inoculated into mice which were treated with the sulfa drug at a determined dosage, and the survival time of the mice was compared with that of control mice.

Results

Effect of acetylspiramycin on toxoplasmosis in mice

In a preliminary experiment, the effect of Asp on toxoplasmosis in mice was examined. All mice of 4 groups as shown in Table 1 were inoculated with 3,000 RH-parasites, and Asp-administration started simultaneously. Among 30 mice which received 8 mg of Asp per day, 6 (20%) survived for 5 weeks after the inoculation, and they were shown to harbor no parasite by subinoculating their brains into clean mice (Table 1). The remaining 24 mice succumbed to RH-infection being demonstrated the organism by microscope examination or by subinoculation. In the mice given 16 mg of Asp daily, 25 out of 30 mice survived for 5 week, and they harbored no parasite. Among 5 mice which died before the 5 th week, 3 were shown to have no parasite by the subinoculation. Thus, in total, the organism was eradicated in 28 (93%) mice by daily anministration of

Table 1 Suppressive effect of acetylspiramycin on the multiplication of RH-toxoplasma in mice

Acetylspiramycin		No. of parasit. inocul.	No. of mice exam.	No. of mice survived					Eradication of organism in mice	
Daily dose	Period of administ.			1 wk.	2	3	4	5	no.	%
8 mg	5 wks.	3,000	30	30	20	7	6	6	6	20
16 〃	5 〃	〃	30	30	28	27*	26*	25*	28	93
40 〃	1 〃	〃	20	20(sacrificed)					3	15
40 〃	2 〃	〃	20	20	20(sacrificed)				20	100
Control		〃	25	14	0				0	0

* Mouse died 3, 4 and 5 weeks of infection were proved to have no parasite, while the other mice died were shown to have parasites by direct examination or subinoculation.

Acetylspiramycin was given daily into the stomach of mouse by tubing.

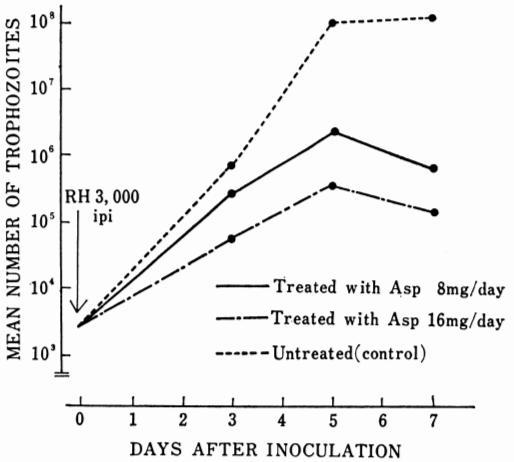
Asp.

After the daily administration of as much as 40 mg of Asp for 1 week, none of 20 mice died in this period, but the eradication of parasite at the end of treatment was shown only in 3 (15%) mice. When the period of the treatment with the same dose was extended to 2 weeks, all of 20 mice could survive and no parasite was detected in any of them. All the control mice died of the infection within 2 weeks.

In another series of experiment which was performed to examine the effect of Asp on the multiplication of the wild type RH-toxoplasmas in the abdominal cavity of mice, 3,000 parasites were inoculated into mice intraperitoneally and the treatment started simultaneously. Number of the parasites per ml of the peritoneal washings was counted on 5 mice each which were sacrificed 3, 5, 6 and 7 days after inoculation. In the case of daily administration with 8 mg and 16 mg of Asp, the mean number of parasites increased gradually and reached the peak on 5 th day showing population of 2.5×10^6 and 362×10^3 respectively, then on 7 th day decreased to 616×10^3 and 142×10^3 respectively (Fig. 1). On the other hand, the parasites in untreated mice multiplied actively showing maximum population of approximately 10^8 on 5 th day, and the population was maintained roughly at the same level until the death of mice.

Form these findings, it is obvious that Asp is able to suppress the multiplication of the parasites resulting in prolongation of survival time of mice or to eradicated the parasites in mice depending on the dosage of the drug used.

Fig. 1 Mean number of parasites detected in 1 ml of peritoneal fluid* after daily administration of acetylspiramycin.

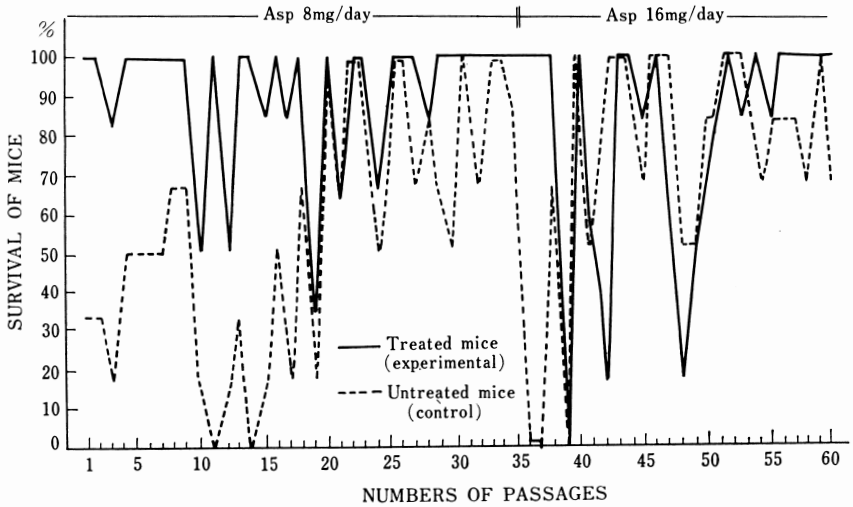


*Counting of parasites was made on the fluid obtained by washing with 2 ml saline injected into the peritoneal cavity of the mice, and the average number indicated were obtained from mice in each sample.

Change in survival rate of infected mice and the number of toxoplasmas in the peritoneal cavities during the passage of Toxoplasma through Asp-treated mice.

As described above, RH strain of *Toxoplasma* was transfered serially through Asp-treated mice, and at each passage a half of the inoculated mice were treated by Asp, while the remaining mice were left without Asp-treatment. The survival rate of each group of mice on 6 th day of the infection was plotted at each passage. The daily dose of Asp was 8 mg per mouse until the 35 th passage and thereafter it was increased

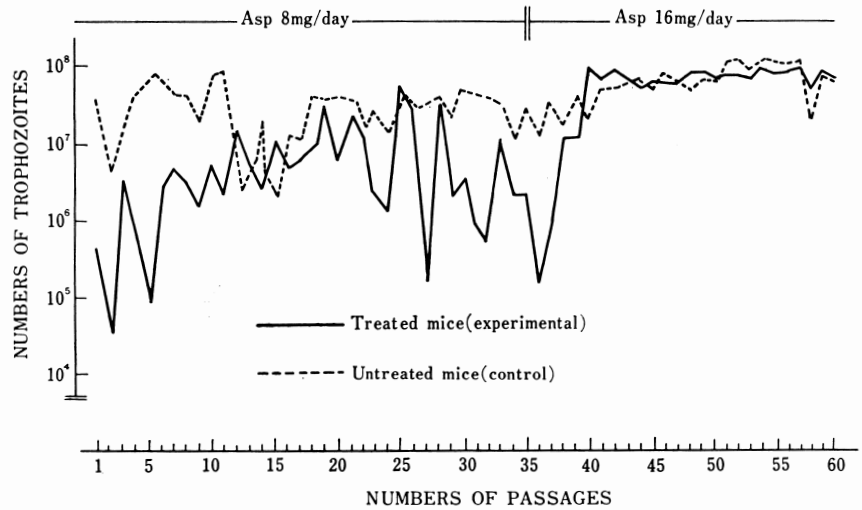
Fig. 2 Survial rate of the mice receiving Asp-treatment daily



Throphozoites harvested from the mice were transfered into abdominal cavity of new mice treated or untreated.

Counting of survial mice was made 6 days after the infection (infection=day o).

Fig. 3 Mean number* of trophozoites detected in the peritoneal cavity of mice receiving Asp-treatment daily



Counting of parasites was performed on the survial mice 6 days after infection.

* This indicates the numbers in 1 ml of abdominal fluid diluted with 2 ml saline.

to 16 mg per mouse.

There was an obvious difference in the survival rate between Asp-treated and untreated mice until 18 th passage showing higher rate in the former group (Fig. 2). Thereafter, until 60 th passage except for 36 th, and 37 th passage, no significant difference in the survival rate was observed between the two groups.

Regarding the number of the organism in the peritoneal cavity of mice examined on 6 th day, Asp-treated mice showed 10^4 to 10^7 parasites in number during 1 st and 11 th passage, while the mice without receiving Asp showed about 10^7 to 10^8 parasites throughout the passages. During 11 th and 37 th passage, the number of parasites in Asp-treated mice fluctuated considerably, but there was a tendency that the number of parasites in Asp-treated mice approximated to that in untreated mice. After 38 th passage, however, number of parasites in Asp-treated mice was kept at the level of 10^8, almost same number as in untreated mice (Fig. 3).

Mean survival time of Asp-treated and untreated mice, which were inoculated with the organisms isolated at 18 th, 20 th, 21 st 25 th and 30 th passage, was shown in Table 2. Among 60 of Asp-treated mice, in total, 58 died of toxoplasmosis within 2 weeks after the inoculation, and the remaining 2 mice died within 3 weeks being the mean survival time of 8.3 days. All the mice untreated died within 2 weeks and the mean survival time was 6.5 days.

The survial time of mice inoculated with the organisms of 40 th, 45 th, 50 th and 60 th passage was observid in the same way. As shown in Table 2, the mean survival time of 60 Asp-treated mice in total was 6.9 days and that of 30 untreated mice was 7.2 days. In these series of experiment, the mean survival time of Asp-treated mice and that of untreated mice were similar.

From these findings, the parasites isolated during 40 th and 60 th passage appeared to be resistant to Asp.

In order to confirm the resistance, another series of experiment was carried out. The mice which were inoculated with, 3,000 trophozoites isolated from 61 st to 100 th passage were examined for survival time to compare with that of the mice infected with the same number of sensitive RH-toxopasmas. As shown in Table 3, all of 240 mice which were infected with resistance strain and received Asp-treatment died out within 2 weeks after infection and mean survival was 5.9 days. On the other hand, 12 out of 20 mice which were infected with sensitive strain and treated with Asp survived the infection for 2 weeks and mean survival time of 8 dead mice was 10.0 days.

Stability of the resistance to acetylspiramycin

Although the parasites obtained from Asp-treated mice at 22 nd passage did not seem to have a sufficient resistance to Asp, the following experiments were performed to confirm this problem. Approximately 10^4 parasites obtained from 22 nd passage-mice were inoculated into 3 mice, and successive transfers of the parasites at the intervals of 3 or 4 days were continued without exposure

Table 2 Survival of mice inoculated with 3,000 parasites which were obtained from determined number of passages through mice receiving the treatment by acetylspiramycin

Passage no.		Daily dose of Asp.	No. of mice exam.	No. of mice survived			Mean survival day
				1 wk.	2	3	
18 to 30	Exp.	8 mg	60	48	2	0	8.3
	Cont.	—	30	11	0		6.5
40 to 60	Exp.	16 mg	60	49	0		6.9
	Cont.	—	30	23	0		7.2

Table 3 Comparison of survival time of mice inoculated with Asp-resistant and Asp-sensitive RH-toxoplasmas under the treatment by Asp

	Organism inoculated	No. of parasit. inocul.	Daily dese of Asp	No. of mice exam.	No. of surviving mice on weeks 1	No. of surviving mice on weeks 2	Mean survival day of dead mice
Exp. 1	* Resistant RH	30,000	16 mg	240	43	0	5.9
Exp. 2	** Sen-RH	〃	16 mg	20	20	12	10.0
Cont.	Sen-RH	〃	—	20	7	0	6.1

* Resistant organism used was isolated from the mice of 61 to 100 passages.
** Sen-RH indicates a wild-type sensitive RH strain.

Table 4 Stability of resistance to Asp after successive transfers of Asp of the parasites into mice without treatment

Organism isolated from passage	No. of passages without treat.	No. of parasites inocul.		Daily dose of Asp	No. of mice exam.	No. of surviving mice in weeks 1	2	3	4	5	Mean survival day of mice
22*	5 to 30	3,000	Exp.	8 mg	48	47	4	1	1	1**	9.2
			Cont.	—	48	31	0				7.1
40*	5 to 100	〃	Exp.	16 mg	160	37	1***	0			6.0
			Cont.	—	160	34	0				5.7
50*	5 to 100	〃	Exp.	16 mg	160	57	0				6.2
			Cont.	—	160	64	0				6.3
60*	5 to 45	〃	Exp.	16 mg	72	4	0				5.4
			Cont.	—	72	9	0				5.4
100*	5 to 40	〃	Exp.	16 mg	64	8	0				6.0
			Cont.	—	64	16	0				6.0
Total (Resistant RH)			Exp.	16 mg	456	106	1	0			6.0
			Cont.	—	456	123	0				5.9

Experimental and control mice were inoculated with the same parasite suspension intraperitoneally.
* Figures indicate the number of passages of parasites through mice receiving the treatment of Asp.
** Parasites in this mouse were shown to be eradicated.
*** This mouse died 15 days after infection, showing a few number of parasites in its abdominal cavity.

to Asp up to 30 th passage. During these passage, 3,000 parasites obtained from mice at every 5 passages were inoculated into 16 mice. One half of the mice inoculated was treated daily with 8 mg of Asp and another half was left untreated. In the case of Asp-treated mice, 44 out of 48 mice died within 2 weeks after the inoculation. Survival for more than 2 weeks was recognized in 4 mice, but 3 of them died within 3 weeks. The remaining one mouse survived for 5 weeks and no parasite was found by subinoculation with its brain into clean mice. On the contrary, all the untreated mice died within 2 weeks. The mean survival time in the Asp-treated and untreated groups was

9.2 and 7.1 days respectively (Table 4).

Toxoplasmas obtained at 40 th, 50 th, 60 th and 100 th passage were sufficiently resistant to Asp. Stability of the resistance was examined by the same way as described above. Parasites which originated from the mice of 40 th passage and thereafter transfered serially 5 to 100 times through untreated mice were inoculated into mice of two groups; one was treated daily with 16 mg of Asp and another was not treated. The total number of mice treated and untreated with Asp was 160 each (Table 4). In case of Asp-treated mice, all the mice died of toxoplasmosis within 3 weeks and the mean survival time was 6.0 days. All the untreated mice also died within 2 weeks and the mean survival period was 5.7 days, showing no significant difference between those of two groups. Similar results were obtained on the parasites from the mice of 50 th, 60 th and 100 th passage.

Table 4 shows the summerized results of survival period of mice inoculated with the organisms which were isolated at 40 th, 50 th, 60 th and 100 th passages. The mean survival time of Asp-treated and untreated mice was 6.0 and 5.9 days respectively, showing no significant difference between both groups.

Virulence of Asp-resistance strain

Inoculation with 1,000 parasites, which were collected from the mice of 51 st, 53 rd, 55 th, 57 th, 60 th and 100 th passage each, was made into the peritoneal cavities of 20 mice. The same number of Asp sensitive RH parasites were inoculated into mice as control.

The mean survival time of Asp-resistant parasite was 7.0 days and that of Asp-sensitive wild type parasite was 6.2 days (Table 5). The mean survial time was consistently extended in every group of mice inoculated with Asp-resistant parasite. Among 20 mice inoculated with the parasites from 60 th passage, one survived for more than 2 weeks and died on 16 th day of the infection, having only a few number of trophozoites in the abdominal cavity. The number of surviving mice 1 week after infection showed the marked difference between both groups; number of survived mice infected with resistant and sensitive parasite was 79 and 43 respectively.

The difference of mean survival days between both groups is statistically significant ($t_0=4.2288>t=2.8334$ ($P=0.005$, $\upsilon=238$)). From these findings, Asp-resistant parasites were shown to have a lower virulence than the wild-type sensitive parasite.

Resistance of parasites originated from a single cell of the resistance strain

Multiplication of the organism in the peritoneal cavity of mice was rccognized by inoculating a single cell into clean mice by the method described above. The parasites multiplied were collected from the peritoneal

Table 5 Comparison of the survivial period of mice infected with 1,000 of resistant or sensitive organisms to acetylspiramycin

Organisms used	No. of mice exam.	No. of surviving mice on weeks			Mean survival day
		1	2	3	
51*	20	15	0		7.4
Sen**	20	13	0		6.8
53*	20	8	0		6.4
Sen	20	4	0		5.8
55*	20	12	0		7.2
Sen	20	3	0		6.2
57*	20	17	0		6.9
Sen	20	1	0		5.9
60*	20	13	1	0	7.4
Sen	20	9	0		6.7
100*	20	14	0		6.9
Sen	20	13	0		6.6
Total					
Resist.	120	79	1	0	7.0
Sensit.	120	43	0		6.2

* These organisms are resistant to Asp and the figures indicate the number of passages of parasites through mice receiving the Asp-treatment.

** Wild type RH-strain was used as a sensitive strain.

Table 6 Stability of resistance of the clones obtained from the Asp-resisant parasites

Organisms used	No. of passages without treat.	No. of parasites inocul.		Daily dose of Asp	No. of mice exam.	No. of surviving mice on weeks 1	No. of surviving mice on weeks 2	Mean survival day
55*	5 to 20	1,000	Exp.	16 mg	32	12	0	6.4
			Cont.	—	32	12	0	6.1
60*	5 to 100	〃	Exp.	16 mg	160	55	0	6.2
			Cont.	—	160	61	0	6.2
80*	5 to 65	〃	Exp.	16 mg	104	57	0	6.7
			Cont.	—	104	57	0	6.7
100*	5 to 35	〃	Exp.	16 mg	56	24	0	6.3
			Cont.	—	56	27	0	6.4
Total		〃	Exp.	16 mg	352	148	0	6.4
			Cont.	〃	352	157	0	6.4

Experimental and control mice were inoculated with the same parasite suspension intraperitoneally.

* These organisms are resistant to Asp and the figures indicate the number of passages of parasites through mice receiving the Asp-treatment.

cavity 10 days later and approximately 10^4 of the parasites were inoculated intraperitoneally into 3 mice. The passage of the parasites was continued at the intervals of 3 or 4 days without treatment by Asp. During these passages, 1,000 parasites obtained from mice in every 5 passage were inoculated into 16 mice. A half of the mice were daily treated with 16 mg of Asp, while the remaining mice untreated.

There was no significant difference in the mean survival time between Asp-treated and untreated mice in each sample (Table 6). The table shows also the cumulative results of survival days of 352 Asp-treated mice and the equol number of mice untreated. The mean survival time of Asp-treated and untreated mice were both 6.4 days.

Therefore, Asp-resistance was retained in the parasites which originated from a single trophozoite and were serially transfered through mice unexposed to Asp.

Resistance of acetylspiramycin-resistant parasite to sulfa drug

Clone of the parasite used was obtained from mice of 100 th transfer being treated with Asp. The parasites isolated were transfered serially through mice without treatment by Asp. Then, 3,000 parasites obtained after 21 passages were inoculated into conventional mice intraperitoneally. Sulfamethopyrazine was administered into the stomach of mice in dosage of 4 or 8 mg daily for 4 weeks immediately after inoculation of parasites and the number of survived mice was observed.

As shown in Table 7, in a case of administration of 8 mg, 10 out of 12 mice survived for 4 weeks, while in 4 mg, 9 out of 12 mice survived for the same period. The mean survival days of the former and latter groups were 26.0 and 20.3 days respectively. These dead mice were proved to have parasites by subinoculation. Including these mice, the eradication rates of parasite in mice treated with 8 and 4 mg were 41% and 8% respectively. On the contrary, all the mice received no treatment of sulfamethopyrazine died of toxoplasmosis and the mean survival period was 7.5 days.

According to above results, it was revealed that Asp-resistant parasite has no cross resistance to sulfa drug.

Discussion

Asp was produced from spiramycin by acetylation at the Laboratory of Kyowa Hakko Kogyo Co. in Japan 1965, and Omura *et al* (1969) demonstrated the chemical structure of spiramycin. Derivative drugs of macrolide antibiotics, such as erythromycin, carbomycin, oleandmycin, leucomycin, spiramycin and others, are generally used in treatment for infectious diseases. Among these macrolide antibiotics, a cross resistance has been sometimes recognized, but usually it has not been completely resistant. Nakazawa *et al* (1965) reported a complete cross resistance of staphylococcus between spiramycin and Asp. Mechanism of antimicrobial action of macrolide antibiotics is believed to be interfence of a protain synthesis in the organism.

Spiramycin and Asp were proved to have antitoxoplasmic effect on experimental and human toxoplasmosis (Garin and Eyles (1058), Cástren (1962), Nakayama and Matsubayashi (1963), Shimizu *et al* (1969), Aoki (1969) and others). In the present study, it was substantiated that Asp has a suppressive effect on the multiplication of parasites resuting in prolongation of the survival period of mice and eradication of parasites sometimes, when administration of Asp started immediately after the inoculation of toxoplasmas.

In the present experiments, Asp-resistant RH *Toxoplasma* was produced by subinoculating the organism serially through mice receiving Asp-treatment. The parasites harvested from 38 th to 100 th passage were proved to be completely resistant to Asp based on the criteria of survival rate of the mice and number of parasites in their abdominal cavities. Furthermore the complete resistance was shown in the clone of resistant parasite. Once acquired, the resistance was invariably maintained for a long time, even after the 100 passage of the parasites through mice without administration of Asp. In general, drug-resistant strain of bacteria have lower virulence than those of sensitive strains. Asp-resistant trophozoites of *Toxoplasma* thus produced were also verified statistically to have lower virulence than that of sensitive as to survival days of infected mice.

Sander and Midtvedt (1971) produced the sulfamethoxazole resistant RH-strain by transfering the orgrnism to mice which were treated by the drug for 290 days; under the treatment by the drug, the survival period of mice which were infected with resistant parasites was significantly shorter than that of mice infected with sensitive RH strain. Among 60 mice infected with these resistant parasites, however, 5 survived the infection for 20 days. Therefore, the nature of this resistance seems to be not complete.

By the cross resistance examination, Asp-resistant parasite did not show any resistance to sulfamethopyrazine. Concerning the mechanism of action of sulfa drug to microorganisms, it is generally accepted that the

Table 7 Sensitivity of Asp-resistant parasites* to sulfa drug**

	No. of Asp-resist. parasites inoculated	Daily dose of sulfa drug	No. of mice exam.	Surivivng period of mice				Mean survival day of dead mice	Eradicat. of parasit. in mice	
				1wk	2	3	4		no.	%
Exp. 1	3,000	8 mg	12	12	12	12	10	26.0	5	41
Exp. 2	〃	4 mg	12	12	12	11	9	20.3	1	8
Cont.	〃	—	6	6	0			7.5	0	0

* Asp-resistant parasites, which were isolated from mice of 100th transfer of parasite receiving Asp-treatment and transfered serially through 21 mice without Asp-treatment, were used for these experiments.

** Sulfamethopyrazine

drug interferes biosynthesis of folic acid. In case of *Toxoplasma*, however, no knowledge has been avaiable on folic acid metabolism.

Summary

Acetylspiramycin (Asp) was proved to have a suppressive effect on experimental murine toxoplasmosis, as shown by prolongation of survival preiod of the infected mice and by eradication of the parasites in the mice, when an appropriate dosage of Asp was administered.

RH toxoplasmas, which were transfered serially through mice treated by Asp in dosages of 8 mg and 16 mg daily, developed Asp-resistance after the 38 th passage. Resistant strain thus produced tolerated the Asp-treatment causing death of mice in short period and active multiplication in the peritoneal cavity of mice inoculated. The Asp-resistance acquired was found to be invariably stable showing its maintenance even after 100 passages through mice without administration of Asp. Asp-resistant parasites were found to have lower virulence than the wild type Asp-sensitive strain.

The parasites originated from a single trophozoite of the Asp resistant parasites were also found to retain the Asp-resistance even after 100 mouse-passages without exposure to Asp. Cross resistance between Asp and sulf drug was not recognized.

Acknowledgement

A part of this study has been reported at the 40 th and 41 st Congress of Japanese Parasitologist (1971 and 1972) and also at the 6 th Singapore-Malaysia Congress of Medicine (1971).

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Toxoplasma gondii のアセチルスピラマイシン耐性株の産生およびその耐性の持続性について

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ヒト寄生原虫の薬剤耐性についてはマラリア原虫を除いて知られていない．本文ではトキソプラスマ (Tp) のアセチルスピラマイシン (Asp) 耐性株の産生を試みた．まづ，Asp の投薬量と投薬期間によつて Tp 感染動物の延命，生残又は Tp 根絶の抗 Tp 作用を認めた．

強毒 Tp 株 (RH) をマウス腹腔内接種と同時に Asp 8 mg/day を胃内に継続注入投与し6日後に次代マウスに継代して，35代以後は Asp 16 mg/day に増量して100 代に及んだ．継代38代以後の継代動物から得られた Tp は Asp 加療マウスの生存日数およびマウス腹腔内の虫体の増殖状況から Asp に完全耐性を示した．これらの耐性株は Asp の投与をうけない無処置マウスへの継代100代後においても，なお，Asp 耐性は持続保持された．これらの耐性株のマウスに対する毒性は感受性株のそれと比して統計学的に有意差を認め，耐性株は僅かながら弱毒化したことを知つた．唯1個の耐性株虫体から出発した虫体についても同様に Asp 耐性の安定性が認められた．

なお，Asp 耐性株は sulfa 剤との間に交叉耐性は認められなかつた．