EFFECTS OF SPIRAMYCINE ON EXPERIMENTAL TOXOPLASMOSIS IN MICE

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Although treatment of toxoplasmosis have been studied extensively by many investigators during these two decades, it remains still unsolved. Despite some drugs protect the death of animal which was experimentally infected with toxoplasma, the organism survives in some tissues of the animals very often being suppressed its multiplication in some extent. This fact is very frequently seen in cases of experimental treatment using mice which is one of the most susceptible laboratory animal to toxoplasma. The mice which were treated with drugs survive the infection having viable toxoplasma in the tissue for a long period.

The present studies were undertaken to evaluate the effects of spiramycine and synergistic effects of spiramycine and pyridazine on experimental toxoplasmosis in mice. Results obtained were compared with those of pyrimethamine, which is considered to be one of the most effective remedies for toxoplasmosis at present time.

Spiramycine was originally extracted from culture filtrates of *Streptomyces ambofaciens* which was isolated from soil in north France by Pinnet Sindico in 1954. This antibiotic is known to be effective, by systemic administration, on several infectious diseases; amebiasis, whooping cough, some virus infections, gonorrhea, some staphylococcus and streptococcus infections, and other diseases.

MATERIALS AND METHODS

Toxoplasma strains used

Beverley strain and RH strain of *Toxoplasma gondii* were used throughout these experiments. Both strains were maintained serially by intraperitoneal inoculations to mice in our laboratory. RH strain is highly virulent to mice,

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killing all of them within one week after inoculation. Beverley strain is less virulent to mice showing a large number of cysts in the brains in chronic stages. Mice weighing about 20 g were used in the experimental treatments as well as in maintenances of the strains.

Drugs used

Because spiramycine is soluble only in acid medium, distilled water added with N/10 H_2SO_4 was used as a solvent. In series A of the experiments, 1600 mg of spiramycine was dissolved in 100 ml of distilled water containing 24% N/10 H_2SO_4 . In series B, 2600 mg of the antibiotic was dissolved in 100 ml of distilled water containing N/10 H_2SO_4 in 38%. For treatments, 0.5 ml of the solutions was administered daily into the stomach of mice using gavages. Therefore, in series A and B, 8 mg and 13 mg of spiramycine were given to individual mouse respectively. Because of the insolubility of pyridazine, 3-sulfanilamido-6-methoxy-pyridazine, the compound was suspended in the acid distilled water and administered orally into mice. Daily dosages was 0.5 ml of the suspension in which 13 mg of pyridazine was contained.

Daraprim was also dissolved in the acid water in concentrations of 50 mg in 84 ml, and 0.6 ml of the solution was given to mice, therefore 0.36 mg of daraprim (roughly equivalent to 18 mg per kg of body weight) was administered to mouse daily. These drugs described above were given into mice for 50 successive days beginning the day of inoculation. In a few cases, the drugs were not given 2 or 3 times during the course of treatment with 1 or 2 weeks interval.

Follow up observation on mice treated

The experimental mice were periodically examined for the presence or absence of toxoplasma in the peritoneal fluid using capillary tubes while they are alive. Mice which survived more than 20 days were sacrificed 21, 30, 40 and 50 days after inoculation and examined for the presence of living organisms in the brains by subinoculating 1.0 ml of the emulsified tissue into the peritoneal cavities of clean mice. The mice which were subinoculated with the brains were periodically examined for the appearance of proliferative form in the ascitic fluid, and they were all sacrificed 21 days after the inoculation to be examined for the presence of cysts in the brains. In addition to the subinoculation of the material into mice, the brains of the sacrificed mice were checked microscopically for presence of cysts. Groups of infected mice, given no treatment at all, were included as controls.

RESULTS

1) Effects of Spiramycine

In cases of RH strain toxoplasma, 10×10^3 to 25×10^3 of the proliferative form, which were obtained from ascitic fluid of infected mice, were inoculated into experimental mice. By oral administration of 8 mg of spiramycine consecutive days, 18 out of 68 mice survived quite actively for 21 days, and on the contrary, all of the control mice, which were infected with the same number of the organism, died out within 8 days, as shown in table 1. On the other hand, administration of 13 mg of the drug prolonged the survival period of the group, showing death within 20 days in 24 out of 46 mice.

In cases of Beverley strain, 30 to 170 cysts which were isolated from the brains of infected mice were inoculated into experimental mice. Administration of 8 mg of the antibiotic resulted in death of 2 mice out of 23 by 21 days after the inoculation, on the other hand, 4 out of 15 control mice died within 21 days. By administering 13 mg of spiramycine daily, 16 out of 17 experimental mice survived more than 21 days, though 4 out of 13 control mice were killed within the same period.

Table 1.
Survival of Infected Mice Treated with Spiramycine

Strain inoculated	Dosage per day	No. of organisms inoculated	No. of mice examined	No. of mice surviving after :		
				10 days	15 days	21 days
RH	8 mg	10-25×10 ³ troph.	exp. 68	38	20	18
			cont. 40	0	—	—
	13 mg	"	exp. 46	40	25	22
			cont. 30	0	—	—
	Total		exp. 114	78	45	40
		cont. 70	0	—	—	
Beverley	8 mg	30-170 cysts	exp. 23	22	22	21
			cont. 15	14	11	11
	13 mg	30-78 cysts	exp. 17	16	16	16
			cont. 13	12	9	9
	Total		exp. 40	38	38	37
		cont. 28	26	20	20	

Treatment was started at the time of infection (day 0) and was continued for 3 weeks.

In the examinations of presence of living toxoplasma in mice which survived more than 21 days, the following results were obtained: In cases of RH strain infection, toxoplasma was found in 15 out of 16 mice which were sacrificed between 21 and 28 days after the inoculation. In mice sacrificed on 30, 40 and 50 days after inoculations, 6 out of 9, 4 out of 6 and 3 out of 5 mice were found

to have living toxoplasma in the brains respectively. In cases of Beverley strain infections, 4 out of 6 mice were found to have viable organisms when they were sacrificed 21 to 28 days after the inoculations. In mice sacrificed 30, 40 and 50 days after inoculation, living toxoplasma was detected in 4 out of 7, 10 out of 10 and 8 out of 13 mice respectively (table 2).

Table 2.
*Presence of the Viable Organisms in the Brains of Infected
Mice Treated with Spiramycine*

Strain inoculated	Dosage per day	No. of organisms inoculated	No. of positive, examined mice after infection			
			21-28 days	30 days	40 days	50 days
RH	8 mg	10-25 $\times 10^3$ troph.	9/10	3/5	2/2	—
	13 mg	"	6/6	3/4	2/4	3/5
	Total		15/16	6/9	4/6	3/5
Beverley	8 mg	30-170 cysts	4/6	1/2	6/6	4/6
	13 mg	30-78 cysts	—	3/5	4/4	4/7
	Total		4/6	4/7	10/10	8/13

Examinations were made by microscope and subinoculations with the brains into clean mice.

Microscopical examinations were performed to find out cysts of toxoplasma in the brains of experimental mice. In cases of RH strain, no cyst was found in all mice. In Beverley strain infections, cysts were found in 10 out of 36 experimental mice in very few number, despite a large number of cysts were found in all control mice.

In one group of mice, administration of the drug was began 3 days after infection. Daily dosage of 8 mg of spiramycine was given to 29 mice which were inoculated with 20×10^3 RH strain toxoplasma, and all of them died 6 to 9 days after inoculation, same as in control mice. Daily administrations of 13 mg of the drug resulted in survivals for 10 days in 4 mice and 21 days in one out of 44 mice inoculated with 10×10^3 to 32×10^3 RH toxoplasmas. In the mouse which survived for 21 days, living toxoplasma was revealed in the brain after subinoculation into clean mice.

Treatment was performed for 10 mice to which a huge number of the organism, 273×10^3 trophozoite of RH strain, was inoculated. Spiramycine was given to the mice in dosage of 8 mg daily, however all of these mice died out within 7 days after inoculation.

As mentioned above, it was found that spiramycine is effective only on

prolongation of survival period of infected mice and certainly not on eradication of toxoplasma even if it was administered in dosage of 13 mg daily for 50 successive days immediately after the infection of the organism.

2) Effects of Combined Use of Spiramycine and Pyridazine

Effects of pyridazine given alone on toxoplasma infections were evaluated preliminary by administrating 13 mg of the drug daily. Among 30 mice which were inoculated with 20×10^3 strain trophozoites, 2 mice died 8 and 9 days after inoculation and the others survived more than 21 days. Toxoplasma was found in 6 out of 28 mice survived. Therefore it was substantiated that pyridazine is partially effective against toxoplasma when it was given in dosage of 13 mg per day for more than 21 successive days.

In order to check the effect of combined use of the two drugs, 13 mg of spiramycine as well as pyridazine were simultaneously administered daily. In 48 mice which were inoculated with 20×10^3 RH trophozoites, all of them survived more than 21 days, inspite of death of 20 control mice within 7 days. Also in Beverley infection, no death was observed in 29 mice, which were inoculated with 18 cysts, by 21 days after inoculations although 6 out of 10 control mice survived more than 21 days (table 3).

Table 3.
Effects of Combined Use of Spiramycine and Pyridazine on Toxoplasma

Strain inoculated	No. of organisms inoculated	No. of mice examined	No. of mice surviving after :		
			10 days	15 days	21 days
RH	20×10^3 troph.	exp. 48	48	48	48
		cont. 20	0	—	—
Beverley	18 cysts	exp. 29	29	29	29
		cont. 10	9	9	6

Spiramycine and pyridazine were given in daily dosage of 13 mg each for 3 weeks at the time of infection

In examinations made on 21, 30, 40 and 50 days after inoculations for detection of viable toxoplasma in the brains of these survived mice, 38 mice which were inoculated with 20×10^3 RH strain toxoplasmas showed no viable toxoplasma with exception of one mouse sacrificed after 40 days. This mouse looked quite healthy before it was sacrificed. By subinoculation with the emulsified brain of this mouse into 3 clean mice, all of these mice died 11 days after the inoculation showing a large number of trophozoites in the ascitic fluid. In cases of Beverley strain infection, none of 28 mice infected showed viable toxoplasma in the brains by subinoculation into clean mice 21 to 50 days after the infection,

The results described above demonstrated that the combined use of spiramycine and pyridazine in daily dosages of 13 mg each for more than 21 successive days was remarkably effective on eradication of toxoplasma in mice experimentally infected. As the shorter period of administration of the drugs seemed to be more advantageous in practical view point, attempts were made to eradicate the organism by simultaneous use of both drugs only for 8 days. Amount of the drugs was 13 mg each per day. Experimental treatment was performed using 20 mice which were inoculated with 25×10^3 of RH strain trophozoites. All of the experimental mice survived for 21 days, and thereafter they were sacrificed and examined for the presence of living toxoplasma in the brains. Toxoplasma was detected in 15 out of 20 mice examined.

As the attempt to treat within the shorter period resulted in failure, effects of administration of the drugs in less quantity in longer period were evaluated in the following experiments. Spiramycine and pyridazine were given 1 mg each per day for 3 successive weeks. After the examinations of living toxoplasma in 7 mice which survived the infection with 22×10^3 of RH strain trophozoites, toxoplasma was detected in all of them by subinoculation.

In order to compare the effects of combined use of spiramycine and pyridazine with those of pyrimethamine, the latter was administered into the stomach of mice with gavage in dosage of roughly 18 mg per kg body weight daily. Period of administration was up to 50 successive days. In 29 mice inoculated with 25×10^3 RH strain trophozoites, no mice died within 21 days and no toxoplasma was detected by subinoculation from the surviving mice which were examined 21, 30, 40 and 50 days after inoculation (table 4). In cases of Beverley strain infection in which 20 mice were inoculated with 184 cysts each, similar results were obtained showing prolongation of survival period and no toxoplasma in the survivals (table 4).

Table 4.
Survival of Infected Mice Treated with Daraprim at the Time of Infection

Strain inoculated	No. of organisms inoculated	No. of mice examined	No. of mice surviving after :		
			10 days	15 days	21 days
RH	25×10^3 troph.	exp. 29	29	29	29
		cont. 10	0	—	—
Beverly	184 cysts	exp. 20	20	20	20
		cont. 10	7	6	3

Daraprim was given in 18 mg/kg/day.

DISCUSSION

It has been supposed that sensitivity of toxoplasma to drugs is different in

strains. Kaufman et al. (1959) stated that 113-CE strain toxoplasma which multiplies more slowly than RH strain, forming cysts like Beverley strain, was less sensitive to pyrimethamine than RH strains in tissue cultures. However, concentration of 5 mg per cent was high enough to kill the organism of both strains. Beverley strain and RH strain were used in the present studies, however, marked differences in sensitivity to the drugs were not recognized in both strains of toxoplasma, so far as the dosages used were concerned. This finding might be caused by the administration of drug in enormous dosages.

Effects of various kinds of antibiotics, chlortetracycline, oxytetracycline, chloramphenicol, etc. against toxoplasma infection have been reported by many investigators. Although it has been recognized that many antibiotics were slightly effective against toxoplasma infection, no antibiotics showed so potent action as to eliminate the organism completely from the tissue.

Garin et al. (1958) reported briefly the effects of spiramycine on experimental toxoplasmosis in mice. By subcutaneous injection of 8 mg of the antibiotic daily for 2 weeks, 8 out of 10 mice survived 42 days after inoculations with 20×10^3 trophozoites of RH strain toxoplasma, showing complete eradication of the organisms from the tissues of mice. In addition, the dye tests became negative in all of the mice treated. In cases of treatment with 4 mg daily for two weeks, 8 out of 10 mice survived for 42 days, however, toxoplasma was detected in 4 out of 8 survivals. These results somewhat disagree with those in the present studies, because viable RH strain toxoplasma was demonstrated from the brains of about 80% of mice which were treated with daily dosages of 8 or 13 mg of the antibiotic for 20 to 50 successive days. Also in Beverley strain infection, similar results were obtained in 72% of mice treated. Differences in results between Garin's experiment and ours might be caused by those in route of administration of the drug. Anyhow, spiramycine, when used alone, is not effective enough to kill toxoplasma in mice tissues, even though it is used in very large doses for long period, starting immediately after the inoculation of the organism.

Eyles et al. (1952) reported the synergistic toxoplasmodicidal action of pyrimethamine and sulfadiazine. By administering both drugs simultaneously, mice inoculated with less than 20×10^3 of RH toxoplasma trophozoites tolerated the infection demonstrating no organisms in the tissues. However, in case of the larger inoculum, the drugs was not effective to kill the organisms completely, despite the survivals of mice were recognized. Similar conditions were observed in the present studies. Inoculation of a large amount of inoculum, 273×10^3 trophozoites, or starting treatment 3 days after the inoculation with the organ-

isms resulted in the earlier death of the mice. According to the above data, it is obvious that the size of inoculum is an important factor which influences the results of experimental treatment. Eyles (1956) obtained similar results in experimental treatment with sulfadiazine.

Complete eradication of the organism was attained by combined use of spiramycine and pyridazine in dosage of 650 mg per kg per day for 21 successive days. As stated above administration of either spiramycine or pyridazine alone at the same dosages did not show so strong killing effects on toxoplasma as comparable with those of combined use of the two drugs. At present time, pyrimethamine is known to be one of the most effective toxoplasmodicides. However, some investigators tend to neglect the high therapeutic effects of pyrimethamine on toxoplasmosis based on their experimental studies in mice. According to Summers (1953) 0.4 mg of pyrimethamine was not effective on preventing the death of mice, while 3 mg or 4 mg, toxic doses of the drug, inhibited the multiplication of the organism. Eyles (1952) also reported that the effect of pyrimethamine on toxoplasmosis was meager, because the MTD/MED ratio was 3 or less. The author's results in the present studies are quite different from those of the above investigators. Differences might be caused by those of method of administration, particularly of duration of administration, consequently total amount of the drugs given.

SUMMARY

Effects of spiramycine and pyridazine were evaluated on experimental toxoplasmosis in mice infected with Beverley or RH strains, comparing with those of pyrimethamine which is known to have potent action to toxoplasma. Spiramycine and pyridazine, when used alone, showed slight killing effects on toxoplasma, although the survival period of mice was prolonged considerably.

Combined use of these two drugs proved effective in oral administration of 13 mg each per mouse, if given immediately after infection for 21 days, demonstrating no viable toxoplasma in almost all mice, experimentally infected.

Therapeutic effects of combinations of these two drugs was almost equivalent to these of pyrimethamine. The two strains of toxoplasma used, Beverley and RH, did not show any differences in susceptibility to these drugs, but this might be due to the fact that relatively enormous dosages of the drugs were administered.

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