[Jap. J. Parasit., Vol. 26, No. 3, 127-131, 1977]

Different Susceptibilities of *Toxoplasma* Strains to Anti-Toxoplasmic Drugs

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(Received for publication; April 4, 1977)

It has been noted on several occasions that the therapeutic effect of an anti-toxoplasmic drug varies considerably depending on each case of toxoplasmosis. Efficacy of drugs on this infectious disease are influenced by various factors such as vilurence of the infecting pathogen, conditions of the host, and the lapse of time after infection. The purpose of this study is to examine another factor, the susceptibility of various *Toxoplasma* strains to anti-toxoplasmic drugs. In this study, eleven strains with a similar degree of vilurence to mice and six anti-toxoplasmic drugs were used.

Materials and Methods

Toxoplasma strain. The eleven strains of *Toxoplasma* used in this study were RH, 9 strains isolated from pigs and one from dog, which were all maintained by serial passage in mice. The names and origins of the strains other than RH were as follows (with place and year of isolation in parenthesis): HG (Tokyo, 1964), TT (Saitama, 1966) from pigs with toxoplasmosis; DP (Tokyo, 1968) from an apparently healthy dog; TA (Tochigi, 1968), OP (Saitama, 1968), 7053 D, 7058 D, and 3431 Li (Niigata, 1969), A-255 B and A-254 (Gumma, 1970) from apparently healthy pigs slaughtered at abattoirs. Of the above strains HG was kindly supplied by Dr. K. Tsunoda, Natinal Institute of Animal Health, Tokyo and the other nine were isolated by us. These strains possessed virulencies in mice comparable to that of the RH strain in terms of survival days after infection.

Drugs. The six anti-toxoplasmic drugs used were SDDS (2-sulfamoyl-4, 4′-diaminodiphenylsulfone) (Ohshima *et al.*, 1967), sulfamonomethoxine (Tsunoda *et al.*, 1966), pyrimethamine (Eyles and Coleman, 1952), spiramycin (Garin and Eyles, 1958), chlortetracycline (Eyles and Coleman, 1954) and clindamycin (McMaster *et al.*, 1973; Araujo and Remington, 1974).

Susceptibility test. Groups of 9-22 female ddY mice, aged 4 weeks and weighing 19-21 g were injected intraperitoneally with 2×10^4 tachyzoites in a volume of 0.1 ml physiologic saline. The inocula were prepared by diluting the peritoneal fluid of infected mice with physiologic saline 3-4 days after infection. The drugs were administered each at 2-4 dose levels by intraperitoneal injection or intragastric incubation in a volume of 0.2 ml per mouse of saline solutions or suspensions prepared with 0.5% CMC in saline. The medication was started 4 hours after inoculation and continued daily for 7 days. The route and the dosage of each drug are indicated in Fig. 1. The drug effect was expressed as the ratio of mean survival days in each experimental group to those in the non-medicated control group, which always fell in the range of 6.5-7.5 days. Survival days more than 30 were defined as 30 days. Additionally, percentages of mice surviving on day 30 and those of completely cured mice as judged by both the absence of cysts under microscopy

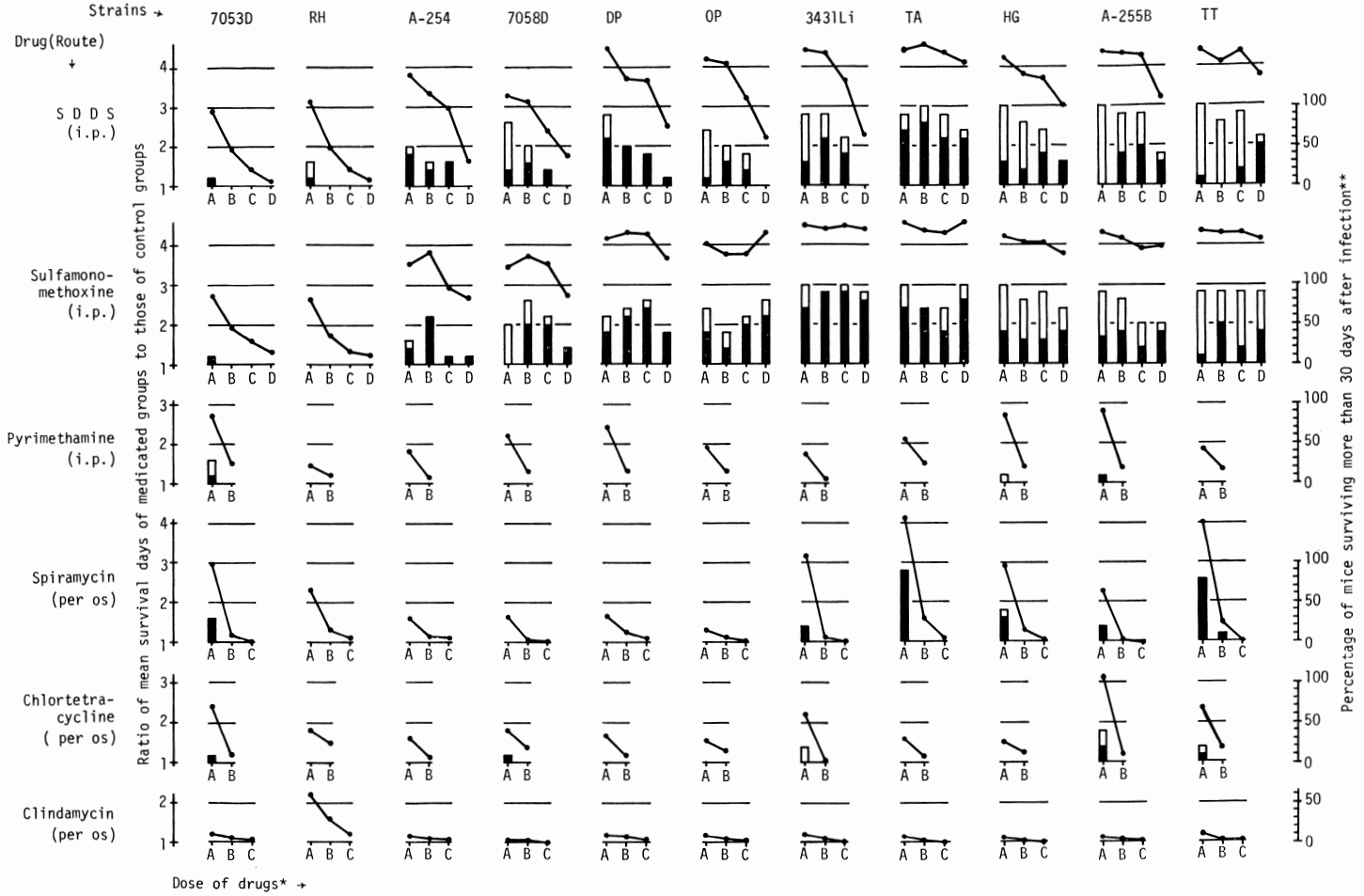

Fig. 1 Comparison of the susceptibilities of 11 *Toxoplasma* strains to anti-toxoplasmic drugs as determined by the drug efficacies in infected mice.

* SDDS and sulfamonomethoxine ; 200 (A), 100 (B), 50 (C) and 25 (D) mg/kg/day, pyrimethamine ; 12.5 (A) and 6.25 (B) mg/kg/day, spiramycin ; 800 (A), 400 (B) and 200 (C) mg/kg/day, chlortetracycline ; 1,000 (A) and 500 (B) mg/kg/day, clindamycin ; 400 (A), 100 (B) and 25 (C) mg/kg/day, each given for 7 days.

** Open columns indicate the percentage of completely cured mice and filled columns indicate the percentage of mice which were positive in the microscopical examination of cysts in the brain and/or in the HA test.

in a portion of the brain and the negative HA titer (less than 1 : 128) of the serum of the mouse obtained with the Toxo-Test kit were determined.

Results

The results obtained in the susceptibility tests are presented in Fig. 1. In the figure the *Toxoplasma* strains are arranged in the order of increasing susceptibilty to SDDS.

The susceptibilities of the eleven strains to SDDS varied considerably from 7053 D to TT. 7053 D killed all the mice given 100 mg or less SDDS/kg/day and, even at the dose of 200 mg/kg/day, 90 % of the mice died, although the mean survival days were 3 times longer than those of the control. In contrast, in the case of TT, 60 % of the mice given 25 mg/kg/day survived for more than 30 days and 60 – 80 % of the mice given 50 mg or more/kg/day were completely cured. The order of the susceptibilities of the strains to sulfamonomethoxine was similar to that of SDDS. To pyrimethamine, 7053 D, HG and A-255 B were the most sensitive strains followed by DP, 7058 D and TA. Spiramycin was most effective with TA and TT and intermediately so with 7053 D, 3431-Li and HG, but only weakly with RH and A-255 B. The most sensitive strain to chlortetracycline was A-255 B, followed by 7053 D, 3431 Li and TT. Clindamycin was significantly effective only with RH, although slight survival effects were seen in mice with some other strains.

The susceptibility pattern to these drugs was considerably characteristic to each strain. For instance, A-255 B and TT were both highly sensitive to SDDS and sulfamonomethoxine and virtually insensitive to clindamycin, but their susceptibilities to pyrimethamine, spiramycin and chlortetracycline showed considerable differences between the two strains. The susceptibility patterns of HG and A-255 B were almost alike, but the two strains differed in the sensitivity to chlortetracycline. RH, which is widely used as a standard strain highly vilurent to mice, had relatively low sensitivities to SDDS, sulfamonomethoxine, pyrimethamine, chlortetracycline, and spiramycin, but it was the most susceptible to clindamycin of the strains used in this experiment.

Discussion

The results from the present study indicate that the susceptibility of *Toxoplasma* to a certain drug is quite variable from strain to strain irrespective of its vilurence, and that each strain posesses a characteristic susceptibility pattern to various drugs. In other words, even if a given drug is highly effective in one case of toxoplasmosis, one cannot assume the same drug to be equally effective in other cases. Catar (1970) reported a remarkable improvement by medication with SDDS in patients with toxoplasmosis to whom pyrimethamine, sulfonamides and spiramycin had been only temporarily or less satisfactorily effective. The difference in therapeutic effect among these drugs may be explained at least partly by the strain difference in drug susceptibility, although the difference in the inherent anti-toxoplasmic activity could not be disregarded.

The above results may be taken as a warning that in evaluating anti-toxoplasmic effects of new compounds there remains a possibility of missing a useful agent unless consideration is given to the strain difference in susceptibility to drugs. Clindamycin has been reported by McMaster *et al.* (1973) and Araujo and Remington (1974) to be effective in murine toxoplasmosis with the RH strain. In our study the same drug did show a significant survival effect on mice infected with RH, but it was only slightly effective on the other ten strains. If this drug had been tested with one of the above ten strains, one would naturally have concluded that clindamycin was practically ineffective.

It is sometimes necessary to identify each *Toxoplasma* strain, for instance, when the infectious source of an outbreak is discussed. We were once asked to determine a *Toxoplasma* strain isolated from a pig which was a survivor of an outbreak of toxoplasmosis

in Kyoto in 1976. The symptoms of the sick pigs were so severe that the disease was once suspected to be a swine fever, but they were successfully treated with SDDD. Two cats which had been caught around the pigpen just after the outbreak were examined for a possibility that these cats might have been the source of the infection. These three *Toxoplasma* strains, one from the surviving pig and two from the cats, could not be distinguished from one another in vilurence to mice since they were all as vilurent as the RH strain. Susceptibility to SDDS was compared among the three strains in the next step. The sensitivitiy to SDDS of the strain from the pig was significantly greater than those from the cats, which excluded the above possibility. The susceptibility to drugs can thus be a valuable marker other than vilurence.

Mechanism or cause of the difference in drug susceptibility among the strains of *Toxoplasma* is unclear. Since the strains employed in the present study had been isolated before the now prevailing use of SDDS and sulfamonomethoxine in large quantities in pigs, it is likely that the difference in susceptibility to these drugs derives from the innate properties of the individual strains.

It is of interest that the order of the susceptibilities to SDDS and sulfamonomethoxine, which are both PABA antagonists, are similar to each other, but unrelated to the order of the susceptibilities to pyrimethamine which affects the adjacent step in the folic acid metabolism.

Summary

Eleven *Toxoplasma* strains with a similar degree of vilurence to mice were tested for susceptibility to six anti-toxoplasmic drugs, SDDS, sulfamonomethoxine, pyrimethamine, spiramycin, chlortetracycline and clindamycin, by efficacy tests in infected mice. It was shown that the susceptibility of *Toxoplasma* to a certain drug was quite variable from strain to strain and that each strain possessed a characteristic susceptibility pattern to various drugs. Significance of the difference in drug susceptibility in therapy, drug evaluation and characterization of *Toxoplasma* strains is discussed.

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トキソプラズマ株間の抗トキソプラズマ剤に対する感受性の差異

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トキソプラズマ症に対する薬剤の治療効果を左右するさまざまな因子の1つとして，感染原虫株の抗トキソプラズマ剤に対する感受性の高低が考えられる．そこで，RH株ならびにマウスに対してRH株と同等の毒力を示す豚および犬由来の10株について，6種の抗トキソプラズマ剤（SDDS，スルファモノメトキシン，ピリメタミン，スピラマイシン，クロルテトラサイクリンおよびクリンダマイシン）に対する感受性を，治療効果を指標として比較した．その結果，トキソプラズマの薬剤感受性は株ごとに可成り差があり，それぞれの株が固有の感受性パターンを示すことを認めた．薬剤感受性の株による差異は治療の上で，また新薬剤を評価する上で考慮すべき問題であり，株のマーカーとしても病原性に次ぐ意義があると思われる．