PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 397-401 (1978)

True and False Prophylaxis in Experimental Trypanosomiasis (40062)

N. ERCOLI

Instituto de Zoología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas

It is generally accepted that, during the period of prophylaxis with chemoprophylactic agents, the host remains protected against the immediate infection, as well as against future infections. However, it has been described how *Trypanosoma congolense* "broke through the protective cover" of prophylactic phenanthridium compounds, inducing an intermittent type of infection in the protected animal; characteristically, these "breakthrough" strains, when transferred to untreated animals, lead to a mortal infection as rapidly as the original trypanosome strain (1). A new diamidine (98/202) of 2-phenyl-thionaphthene (2), in subcutaneous doses of 100 mg/kg protected mice inoculated 20 days later with multiple lethal doses of *Trypanosoma venezuelense*; when the reactivity of the protected mice to reinoculation was tested 1 1/2 months later, they developed an intermittent parasitaemia beginning the next 3–4 weeks, followed by death (3). The considerable delay in the development of the parasitaemia and its intermittent form we attribute to a residual prophylactic action of the drug, with the corollary that the inoculum injected during an assumed period of protection may develop much later into a lethal infection. This hypothesis that the inoculation may cause a latent infection in the presence of the prophylactic drug without manifesting a detectable parasitaemia, and that the chemotherapeutic prophylaxis is not an all-or-none phenomenon, has been investigated.

Materials and methods. T. venezuelense, (T. evansi), isolated by Dr. Raul Hernández from a case of equine "derrengadera" in 1969, was maintained since through mouse passages in our laboratory and used for various chemotherapeutic studies. Its virulence for mice and rats was repeatedly titrated: inocula above 100 parasites killed 95% of the injected animals in a time interval (7–16 days) varying according to the heaviness of the inoculum. The standard ip inoculum of 5×10^5 parasites, as used in the present study, induced detectable parasitaemia within 24 hr in 63 ± 3%, within 48 h in 90 ± 2%, within 72 hr in 94 ± 1.6% of mice. The progressive parasitaemia terminated in death 3–6 days after its appearance. The rats were as sensitive as mice to the standard inoculum containing 5×10^5 trypanosomes: parasitaemia appeared in 96 ± 1% of the animals within 24–72 hr, increasing progressively until death which occurred 1–7 (av. 5.4) days later.

In the chemoprophylactic experiments, the sc injection of these drugs to groups of 14–35 mice or rats was followed at various periods by the ip inoculation with 5×10^5 trypanosomes. During the first period (2–3 weeks) after inoculation, daily observations were made, if the animals showed no parasitaemia during this interval, the observations were reduced to alternate days.

The infectivity of a selected number of mice and rats was tested by transferrring blood, and spleen and liver macerates suspended in saline, to groups of 4–5 mice. Occasionally, after a certain period of clearance the animals were reinfected with the standard inoculum and further observed for the development of parasitaemia.

Results. Continuous observation of the infection in 95 mice pretreated with 98/202 (five experiments) revealed that the parasitefree period is drug concentration dependent, since it varied as a function of the dosage if injected at the same time before inoculation, and, when the same dose was given, it decreased with the interval from the pretreatment. Thus, groups of mice inoculated 20 days after pretreatment with 100 mg/kg 98/202, remained parasitefree for 106–121 days, for 26–41 days with 75 mg/kg, and for 10–13 days with 25 mg/kg; inoculation 55 days after pretreatment demonstrated that 75 mg/kg and 25 mg/kg of 98/202 did not retard the appearance of the parasites, although the treatment often delayed their multiplication and the time of death (Table I). Four mice inoculated 8 days after pretreatment with 12

0037-9727/78/1573-0397$01.00/0

mg/kg 98/202 remained parasitefree for 8–14 days and died 17–24 days after inoculation; with the same pretreatment, the inoculation after 25 days did not alter significantly the first appearance of the parasitaemia, yet it depressed its progression rate and in 2/4 animals delayed by 10 days the time of death compared to the controls (which died in 7–8 days). The latent infection has been confirmed in a number of experiments by the infectivity of the organs (spleen, liver, blood) of apparently parasitefree animals. The virulence of the parasites transferred from the intermittent infection following the parasitefree period was not modified in comparison to the strain used for inoculation.

Similar results, even though the clearing period remained shorter, were obtained with pentamidine in four experiments including 108 mice. Doses as high as 75 mg/kg injected 6 days before the inoculation of the trypanosomes maintained the majority of the animals parasitefree for 13–26 days, after which the infection ran its fatal course. In 10 mice inoculated 7 days after pretreatment with 40 mg/kg pentamidine, the appearance of parasitaemia was delayed by av. 4 days (min. 0; max. 10) compared to the not pretreated controls. The progression of the parasitaemia until death was slowed down also: the controls died in 5–6 days; the pretreated ones, in av. 13.5 days (min. 13; max. 20). Inoculation 10 days after pretreatment with 40 mg/kg pentamidine, delayed the appearance of the parasites in 1/10 mice only; 6/10 died with a delay of 9–11 days. Table II illustrates an experiment with pentamidine. These phenomena became magnified by using rats for the prophylactic treatment with pentamidine: inoculation 6 days after treatment with 20 mg/kg gave the impression of prophylaxis for 37.6 days, which by inoculation 11 days after pretreatment was reduced to 26.2 days (Table III). In one rat of the former group (No. 170) the intermittent infection was detected only in a single observation made 49 days after inoculation; this rat reinoculated 26 days later, developed a progressive infection after 3 weeks, which counted from the day of pretreatment, represents a protection of 3 months duration.

TABLE I. FALSE PROPHYLAXIS WITH 2-PHENYL-THIONAPHTENE DIAMIDINE (98/202) IN THE *Trypanosoma venezuelense* INFECTION OF MICE (SAME DOSE, DIFFERENT INTERVALS; EXP. No. 8).

98/202 (mg/kg, sc) Day 1	Mouse No.	Inoculation of *T. venezuelense* on day	First appearance of parasitaemia (days after inoculation)	Died
75	199	9	38	46
75	201	9	24	45
(Controls) 0	213–224	9	1;2	3;4
75	203	23	25	41
75	204	23	22	61
(Controls) 0	251–252	23	1;2	4;4
75	211	55	2	24
75	213	55	1	2
75	214	55	2	7
75	215	55	1	3
(Controls) 0	290–291	55	1;2	4;5

However, pentamidine and 98/202 as well as other diamidines possess high clearing action without curative effect in the *T. venezuelense* infection of mice and these observations could be attributed to the specific nature of the drugs, rather than to the manifestation of a general phenomenon. In the chemotherapeutic experiments, by sc injection of the drugs 48 hr after inoculation, the clearing dose (ED_{50}) was 1.25 mg/kg for 98/202, 1 mg/kg for pentamidine, and 0.7 mg/kg for suramin. The diamidines, as reported for the antimonials (4), induced no cure by single administration, which was obtained with suramin, 15 mg/kg being its CD_{50} (unpublished). Therefore, the latter drug with definite curative action against this infection, has been investigated for its prophylactic prop-

TABLE II. FALSE PROPHYLAXIS WITH PENTAMIDINE IN THE *Trypanosoma venezuelense* INFECTION OF MICE.

Pentamidine mg/kg subcut. Day 1	Inoculation *T. venezuelense* Day	Trypanosomes appeared		No. died inoculation [av. (min.–max.)]
		In No. of mice	Days after [av. (min.–max.)]	
37.5	3	14/14	15 (6–26)	26 (16–35)
37.5	6	8/8	15 (7–19)	22 (15–28)
75.0	6	4/7*	21 (19–26)	32 (24–40)
Controls		5/5	1.3 (1–2)	5.4 (5–7)

[a] The ultimate fate of 3/7 mice negative on day 40 was not determined.

erties by various pretreatment schedules in mice (Table IV). It is obvious that, according to the drug concentration in the host at the period of the inoculation, the prophylactic action may remain temporary, with variable, often very long delay in the development of the infection, or else result in the complete destruction of the parasites.

With suitable dosage schedule of suramin pretreatment, a protracted period of false prophylaxis was obtained in rats. Pretreatment with 5 mg/kg 7 days before inoculation, delayed in 4/6 rats the appearance of parasitaemia by av. 21 days (min. 11; max. 25) and

TABLE III. PROPHYLACTIC EFFECT OF PENTAMIDINE AGAINST *Trypanosoma venezuelense* IN RATS.

Exp. No.	No. rats	Pretreatment (mg/kg, sc, of pentamidine)		Inoculation (days)	First appearance of parasite Death of animal . . . day after inoculation				
144	2	Controls	—	(5)	2	2			
					7	7			
	3		20	5	19	32	[a]		
					34	44			
	2	Controls	—	(13)	<3	<3			
					8	8			
	3		20	13	23	25	[b]		
					29	30			
145	5	Controls	—	(6)	2	4	2	2	2
						N.D.*			
	5		20	6	27	28	40	44	49
					43	40	54	50	
	5	Controls	—	(11)	2	3	2	2	3
					6	7	7	7	9
	5		20	11	23	23	24	25	36
					32	39	50	36	43
152	3	Controls	—	(3)	1	1	1		
					6	6	7		
	4		10	3	3	17	17	7	
						N.D.*			
	3	Controls	—	(9)	<2	<2	<2		
					3	2	2		
	4		10	9	2	<2	<2	<2	
					3	2	3	3	

[a] One of three rat parasitefree during 70 days observation.
[b] Blood transfer of 1/3 rat 28 days after inoculation negative: animal cured.
[c] Rat 170 (see text): parasites appeared only 1 day; reinfected 75 days after first inoculation became positive in 20 days, i.e. 101 days after pretreatment.
* N.D. Not determined; infected animals used for different experiments.

TABLE IV. TRUE AND FALSE PROPHYLAXIS WITH SURAMIN IN THE *Trypanosoma venezuelense* INFECTION OF MICE.

Suramin (mg/kg sc) Day 1	Inoculation of *T. venezuelense* (day)	Trypanosomes appeared		Prophylactic effect
		In No. mice	Days after inoculation	
Controls (0)		20/20	2	
1.25	10–15	10/10	2–3	None
2.5	7–15	15/16	4–5	None
5.0	7	5/6	13–41	False in 15/26
5.0	10	8/15[a]	24–45	True in 11/26
5.0	15	2/5	10–24	
10.0	7	1/5	60	True in 4/5[b]
20	7	0/5		True (b)

[a] A strain isolated from mouse No. 2495 manifesting parasitaemia 28 days after inoculation is fully sensitive to Suramin.
[b] Transfer of blood and organs induces no infection.

the time of death by av. 33 days (min. 29; max. 44); 2/6 rats remained parasitefree during 44 days observation and were reinoculated: one developed a progressive lethal infection 4 weeks later (102 days after treatment), the other died, without showing parasitaemia. Postponing inoculation to 11 days after 5 mg/kg suramin treatment, in 1/5 rats the infection was not modified; in 3/5, an intermittent infection developed after 23–44 days, leading to death 20–64 days later. In 1/5 rats a progressive infection started 28 days after inoculation, terminating with death 9 days later. Pretreatment with 2.5 mg/kg suramin followed by inoculation 7 days later, only delayed (4–6 days) in 10/14 rats the appareance of parasitaemia with an intermittent infection in 2/10. These 10 rats died in av. 14.5 days (min. 10; max. 21), compared to 9.7 days (min. 7; max. 13) of the control animals. The virulence and the suramin sensitivity of strains recovered after the temporary protection were not modified.

Discussion. The lack of drug sensitivity and virulence variation of the parasites recovered during or after the period of false prophylaxis minimizes the possibility of biological modifications in the trypanosomes and allows an essentially pharmacological interpretation. The prophylactic actions described can be classed in the following categories: (a) true prophylaxis, total protection, without reappearance of the infective organism, and without infectivity of the blood or organs to other animals; (b) false prophylaxis, spontaneous reappearance of the infection at varying periods after inoculation, time of reappearance being a function of the dosage and time of infection, together with infectivity of the blood and/or organs to other animals; (c) with effective prophylactic drugs at certain concentration levels, both effects may appear in a group of animals; thus, one might determine the "True prophylactic $Dose_{50}$". Presumably, the parameters of the prophylactic effect are represented by the antitrypanosomal action of the drug *per se*, by the drug concentration in the body at time of inoculation, and by the half life of the drug.

The intermittent development of the infection after its delayed appearance is no doubt related to the presence in the host of residual drug, which though insufficient for total control of the parasitaemia, still has a moderating effect on its rate of development. Probably only when the drug is totally eliminated does the progressive, lethal course of parasitaemia begin. Circumstantial evidence for the supposition that the reappearance of parasitaemia in the prophylactically protected animals is related to the falling drug concentration consists in the correspondence of the period of the reappearance with that of the termination of the protective effect against inoculation (e.g., 7–8 weeks with 75 mg/kg 98/202). At least semantically, the dose and concentration ranges leading to true prophylaxis, which do not allow the survival of the trypanosomes of the heavy inoculum, may be classed as "trypanosomicidal", while those resulting in false prophylaxis are "trypanosomostatic" since they only delay the development of the inoculum without destroying it. This classification does not necessarily represent the immediate mechanism involved, since adjuvant immunological processes and the concentration/time/effect factors are also involved in the final anti-parasitic action. Thus, in prophylaxis, as in the chemotherapeutic process itself, the drug may have a clearing action, with temporary disappearance of the parasitaemia, often accompanied by an intermittent infection, or else a radical suppressive effect, depending on the nature and dosage of the drug. It is justified to ask to what degree these phenomena are related: our observations in chemoprophylaxis (delay, nonpatent period, intermittency) recall phenomena noticed using subcurative doses in chemotherapy. The duration of the therapeutic clearing effect is dose dependent and possibly, some retention of the drug in the organism may be one of its determinants; this is certainly the case with temporary protection obtained by prophylactic treatment. It would be worthwhile to investigate the problem of the therapeutic clearing effect and of the false prophylaxis in terms of the available and stored drug concentration in the host.

The epidemiological significance of the false prophylaxis is obvious, and its possible extent in other infections, particularly under field conditions, should be taken into account.

Summary. Prophylactic treatment of *Trypanosoma venezuelense* infections in mice

may induce qualitatively different effects. Certain agents such as the diamidine of 2-phenyl-thionaphthene (98/202) and pentamidine with limited curative effect against the infection, in protected mice maintained a long parasitefree period, followed by an intermittent type of infection terminating in death. The therapeutically highly effective suramin, administered before inoculation in sufficient doses, gives a complete "true" protection against the infection; however, if the drug concentration in the host is reduced at the time of inoculation, either by diminishing dosage or by delaying the time of inoculation, the protection becomes only apparent. This "false" prophylaxis consists in a nonpatent (20–40 day) period during which parasites were not seen in the blood, followed by the intermittent parasitaemia with lethal result. In a similar group of animals, appropriate dosage schedules may produce either effect, the true or the false prophylaxis.

Study supported by the Consejo Nacional de Investigaciones Científicas y Tecnológicas de Venezuela (CONICIT) grant S1-0111. The valuable assistance of Ms. Marisol Bolivar, Ms. Morella Rodríguez and Ms. Nelly Olivo is gratefully acknowledged. We are indebted to Professor Otto Dann for samples of his preparation 98/202 and to May and Baker, Ltd. for pentamidine.

1. Hill, J., Ann. Trop. Med. Parasitol. **56**, 426 (1962).
2. Dann, O., Liebigs Ann. Chem. **734**, 23 (1970).
3. Ercoli, N., and Bolívar, M., Acta Cient. Venezolana **24**, (Supl. 1), 62 (1973) = Abstract.
4. Ercoli, N., Villaroel, G., and McLure, M. T., Proc. 8th Intern. Congress Chemother., Athens, **1**, 1012 (1974).

Received July 17, 1977. P.S.E.B.M. 1978, Vol. 157.