PRELIMINARY AND SHORT REPORT SECTION

EFFECT OF STILBAMIDINE THERAPY ON EXPERIMENTAL *BLASTOMYCES DERMATITIDIS* INFECTIONS IN MICE*

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Recent reports in the literature have indicated that the use of stilbamidine (4,4′-diamidinostilbene diisethionate) in clinical cases of blastomycosis has been measurably effective (1, 2, 3). Heilman, using an experimental blastomyces infection in mice, showed that stilbamidine exerted a suppressive effect on the pulmonary infection caused by intravenous injection of *Blastomyces dermatitidis* (4). A report by Schwarz and Adriano (5) indicates that experimental therapy with stilbamidine against blastomyces infections in mice was ineffective. It is believed that experiments carried out in this laboratory on the effect of stilbamidine against experimental infections of *B. dermatitidis* in mice may serve to correlate these two varying points of view.

METHODS AND MATERIAL

A series of experiments was run with four-fold dosage increments of stilbamidine as therapy for blastomyces-infected mice and in uninfected mice for toxicity control in order to titrate the amount of compound needed to protect 50% of the infected mice (PD_{50}), and to determine the amount that would be toxic for 50% of the uninfected mice (TD_{50}). All solutions of stilbamidine used for treatment were aqueous, made fresh daily in order to eliminate the possibility of deterioration of the material. Ten Carworth Farms (CFI) female albino mice, 14–16 gms., were used on each dosage level and for controls. The mice to be infected were given an intraperitoneal injection of 0.5 ml. of a 10^{-2} suspension in 5% mucin of *B. dermatitidis* taken from a five day blood agar yeast phase culture which had been washed with saline. The cells were resuspended in saline and adjusted to a Klett-Summerson Colorimeter reading of 300 with a green filter. Treatment was begun immediately at the time of infection with 0.1 ml. intraperitoneal doses of stilbamidine and continued once daily thereafter for a total of eleven doses. All mice were observed for at least forty-two days and survivors were then sacrificed and observed for gross pathology.

RESULTS

The intraperitoneal toxicity of stilbamidine gave a TD_{50} of .839 mg. per dose by the Reed & Muench method (6). Levels of 5.0 mg. per dose, 1.25 mg., 0.312 mg., and 0.078 mg. were given daily for eleven days. The 5.0 mg. mice died within an hour after the first injection and seven of the mice that received 1.25 mg. died within one day. All other mice survived through the forty-second day, when they were discarded. Table I shows the distribution of deaths from toxicity and the mean survival times for each level of the compound.

A protection experiment, run in four-fold dilutions at lower levels, resulted in a PD_{50} of 0.06 mg. according to the method of Reed and Muench (6). The dosage levels began at 0.5 mg. per dose to 0.007 mg., one dose daily for eleven days. At the end of the forty-two day observation period, the PD_{50} was calculated and the surviving infected animals were autopsied.

It will be seen by the mean survival times listed in Table II that even at the lower doses where the treatment was not sufficient to protect the mice, the increase in the mean survival times was quite marked. Toxicity control mice receiving 0.5 mg/dose on this same experi-

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Received for publication January 12, 1954.

TABLE I

Stilbamidine toxicity

MG/DOSE	SURVIVALS/TOTAL	MST	
0.078	10/10	>42	
0.312	10/10	>42	TD_{50} = .839 mg.
1.25	3/10	16.4	
5.0	0/10	<1.0	

TABLE II

Stilbamidine therapy

TREATED	S/T	MST	AUTOPSY OF SURVIVING MICE AFTER 42 DAYS	
0.007 mg.	3/10	31.7	3 Normal	
0.031	1/10	32.2	1±	PD_{50} = 0.06 mg.
0.125	7/10	39.6	5 Normal; 2±	
0.5	0/10	9.8		
Control	0/10	17.0		

ment showed 100% survival at this level; however, these figures may be reconciled, as explained in the Discussion. Autopsies performed on the mice surviving the infection through treatment were graded according to the gross pathology. Animals that had no gross evidence of caseation were rated as being normal. Single small caseated nodules were rated as being ±. In no animal was there found any evidence of extensive infection.

In another experiment in which frequency of dosage was compared, stilbamidine given intraperitoneally for nine days twice a day showed no difference in protection from stilbamidine given intraperitoneally once a day for eleven doses.

DISCUSSION

Stilbamidine therapy has been successful in this laboratory against experimental *B. dermatitidis* infections in mice. Protection has been found in a preliminary experiment when the dose was as low as 0.0625 mg. per day if the treatment was started at the time the infection was given. It has been observed that in an intraperitoneal infection of the type used in this laboratory, the first gross signs of pathology, caseated nodules in various portions of the peritoneal cavity, may be seen as early as the fourth day after infection, so that the disease must be firmly established and disseminated throughout the body even earlier. The mean survival times of the controls in our experiments have been usually from 15–17 days with deaths from 8–25 days following intraperitoneal infections, while death from an intravenous infection occurs several days earlier. Under these conditions, subcutaneous treatment begun the eighth day after intravenous infection, as done by Schwartz (5), would be unlikely to show the therapeutic value of the drug, as the infection would be well entrenched and almost at its terminal point before treatment was begun. Even when treatment is instituted at the same time that the animals are infected, late deaths may be expected to occur, since it is seldom that 100% of the animals treated are sterilized. The prolongation of life with stilbamidine therapy is indisputable in the treated animals, as evidenced by the mean survival times, and a majority of the animals may be considered cured, if the lack of pathology upon autopsy may be used as an index. Since a toxicity level is run simultaneously with the treatment level, there is no danger of confusing deaths from infection with toxicity deaths. At the border-line toxicity levels deaths may be obtained in the infected treated mice where the uninfected treated (toxicity) mice survive. Presumably border-line toxicity is further enhanced by the added stress of infection.

It is dangerous to attempt to translate the activity of any therapeutic agent in the laboratory animal into terms of efficacy in the human patient, as the response of these two hosts may vary widely, but the experimental response may be used to indicate the clinical approach. Under the test conditions in this laboratory stilbamidine is of considerable value in the treatment of experimental *B. dermatitidis* infections in mice, and in spite of the reported toxicity in humans, would appear to be worthy of further study in the clinical case of blastomycosis. Several authors, including Heilman (4) and Snapper (7), have expressed an opinion that 2-hydroxystilbamidine will have the therapeutic value of stilbamidine with somewhat lessened toxicity. Comparative studies of these two drugs are in progress in this laboratory and will be reported at a later date.

SUMMARY

A report is given of experimental therapy with stilbamidine against a *B. dermatitidis* infection in mice. Female albino mice were infected intraperitoneally with a yeast phase suspension of *B. dermatitidis* in mucin and treated intraperitoneally with stilbamidine daily for eleven days. The TD_{50} was 0.839 mg. per dose and the PD_{50} was 0.06 mg. per dose. Further study of stilbamidine as a possible agent for the treatment of blastomycosis in humans appears to be justified.

We would like to express our appreciation to the William S. Merrel Company for supplying the stilbamidine and 2-hydroxystilbamidine used in these experiments.

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