THE EFFECT OF DI-PARALENE (CHLORCYCLIZINE HYDROCHLORIDE) ON SYSTEMIC FUNGOUS INFECTIONS IN MICE*

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In the past fifteen years, far reaching advances have been achieved in the field of chemotherapy. Many of the bacterial and rickettsial diseases today respond to one or more of the newer chemotherapeutic agents or antibiotics. On the other hand, the clinical course of most of the systemic fungous diseases is not altered by the present antibiotics. Although the antibiotics have not been shown to have a potentiating effect upon fungous diseases, the common complication of moniliasis following antibiotic therapy has been observed by a number of investigators (2–4). Hammer, *et al.* (1) compared the *in vitro* activity of a number of the antibiotics and potassium iodide against a large group of fungi. The authors concluded that potassium iodide had no marked effect on fungous growth *in vitro* even in concentrations higher than could be achieved in blood levels. Of the antibiotics tested *in vitro*, chloromycetin, neomycin, and terramycin showed greater activity than aureomycin, bacitracin, fumagillin, penicillin, or streptomycin.

In this laboratory it has been demonstrated (5) that Di-Paralene is effective *in vitro* against many of the fungi causing systemic mycosis. In view of these observations and the low toxicity of the drug (6) studies on its *in vitro* effectiveness against *Candida albicans* and *Cryptococcus neoformans* infections were instituted.

MATERIALS AND METHODS

Twenty-one day-old male white mice were used throughout these experiments. The mice were inoculated by the intraperitoneal route, and cultures of the lungs, spleen, liver, and kidneys were made from the animals that died spontaneously on or before the 45th day after inoculation. The cultures were made on Sabouraud's glucose agar containing 30 units of penicillin and 30 units of streptomycin per milliliter. The cultures were incubated at 37°C.

The role of gastric mucin in lowering host resistance to fungous infections has been clearly shown by a number of investigators (7–9). The five per cent gastric mucin was prepared as follows: Granular mucin (Type 1701–W, Wilson Laboratories) was suspended in a Waring blendor to a concentration of 5% in distilled water. After standing for 24 hours, the pH was adjusted to 7.2 with one normal sodium hydroxide and the suspension filtered through cheesecloth. The mucin suspension was intermittently sterilized, 15 minutes at 10 pounds pressure, on three successive days. The five per cent mucin was mixed with an equal amount of fungous cells suspended in sterile saline prior to animal inoculation.

The strain of *Candida albicans* used was isolated from a case of generalized cutaneous moniliasis, and the strain of *Cryptococcus neoformans* was isolated from a fatal case of torula meningitis. Both cultures were maintained in the laboratory on Sabouraud's dextrose agar slants.

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The cells utilized for inoculation were grown in flasks of Sabouraud's maltose broth for 48 hours at 37°C. Following this period of incubation the cells were washed three times in sterile saline and finally resuspended in a sufficient volume of mucin solution to give the desired number of cells per milliliter. The number of cells was determined by means of a Spencer hemacytometer. Plate counts were not made because the cells tend to form groups making such counts inaccurate.

Varying concentrations of Di-Paralene were prepared in distilled water and the resulting solution autoclaved for 20 minutes at 15 pounds. Experimental mice receiving the drug or control mice receiving sterile saline solution, were injected with 0.1 ml. daily for 45 days by the subcutaneous route in the groin region. Mice were held 15 days beyond the termination of the experiment to observe any deaths which might occur following the cessation of drug therapy.

Mice that died within 48 hours after infection were not included in the data. Such deaths were assumed to be due to trauma or other causes rather than the effects of the specific infection.

RESULTS

For experiments I and II, *C. neoformans* was employed as the test organism. The mice were infected with approximately 1×10^6 cells suspended in a gastric mucin. Group A mice received 0.25 mg. of Di-Paralene (10 mg./kg.) daily. Group B received 0.375 mg. (15 mg./kg.); Group C, 0.5 mg. (20 mg./kg.); and Group D, 0.675 mg. (25 mg./kg.). There were two control groups; one non-infected which received a single injection of gastric mucin solution followed by daily injections of 0.675 mg. of Di-Paralene, the other infected which received 0.1 ml. of sterile saline daily. The data from experiments I and II is incorporated into Table I as the two experiments were comparable, differing only in the time of the drug being instituted.

Only 30 per cent of the control group died, thus giving doubtful significance to the findings with the test group. The results must be considered inconclusive, although there was some evidence to indicate that there was a greater mortality rate in the groups of mice receiving Di-Paralene.

In a second series of experiments *C. albicans* was employed as the infecting organism. Twenty-one day-old white mice were inoculated intraperitoneally with 8×10^6 cells suspended in mucin. Two levels of Di-Paralene were used; 0.625 mg./mouse (25 mg./kg.) and 0.25 mg./mouse (10 mg./kg.).

Table II represents a composite of four experiments using *C. albicans* as the infectious agent. The mortality in the control group of 54.5 per cent indicated that the strain of *C. albicans* employed was of lower pathogenicity than the isolate 3148 employed by Salvin, *et al.* (9). The *Candida* strain used by us produced fewer toxic deaths within the first week of infection than that used by Salvin, *et al.* The number of deaths occurring among our experimental mice during the first seven days of infection never exceeded five per cent as opposed to the 20 per cent reported by other authors (9).

There appeared to be a lowered mortality rate in the mice which received the drug one and two days prior to infection, but due to the small numbers of mice employed, the figures do not have statistical significance. Mice receiving the drug one and two days post infection showed a marked increase in mortality in

TABLE I

Effect of Di-Paralene on C. neoformans infections in mice

GROUP NUMBER	MG DRUG/DAY	DATE DRUG STARTED	MORTALITY*	% MORTALITY
Group A-1	0.25	2 days pre-infection	6/10	60
Group A-2	0.25	day of infection	3/10	30
Group A-3	0.25	1 day post-infection	4/10	40
Group A-4	0.25	2 days post-infection	4/10	40
Group B-1	0.375	2 days pre-infection	4/10	40
Group B-2	0.375	day of infection	2/10	20
Group B-3	0.375	1 day post-infection	7/10	70
Group B-4	0.375	2 days post-infection	4/10	40
Group C-1	0.5	2 days pre-infection	3/10	30
Group C-2	0.5	day of infection	5/10	50
Group C-3	0.5	1 day post-infection	5/10	50
Group C-4	0.5	2 days post-infection	2/10	20
Group D-1	0.625	2 days pre-infection	6/10	60
Group D-2	0.625	day of infection	5/10	50
Group D-3	0.625	1 day post-infection	4/10	40
Group D-4	0.625	2 days post-infection	6/10	60
Non-infected controls E	0.625	Started same time as Group A-1, B-1, C-1, and D-1	10/20	0.0
Infected controls F	0.1 ml. sterile saline daily	Started same time as Group A-1, B-1, C-1, and D-1	6/20	30
		Total no. of mice	200	

* The numerator indicates the mortality and the denominator the total number of mice on experiment.

TABLE II

Effect of Di-Paralene on C. albicans infections in mice

GROUP NUMBER	MG. DRUG/DAY	DATE DRUG STARTED	MORTALITY*	PER CENT MORTALITY
Group A-1	0.25	2 days pre-infection	2/10	20
Group A-2	0.25	day of infection	3/10	30
Group A-3	0.25	1 day post-infection	41/50	81.8
Group A-4	0.25	2 days post-infection	29/48	60.5
Group B-1	0.625	2 days pre-infection	2/10	20
Group B-2	0.625	day of infection	3/10	30
Group B-3	0.625	1 day post-infection	28/50	56.2
Group B-4	0.625	2 days post-infection	31/50	62
Non-infected Controls C	0.625	Started same time as Group A-1 and B-1	0/29	0.0
Infected Controls D	0.1 ml. sterile saline daily	Started same time as Group A-1 and B-1	24/44	54.5
		Total no. of mice	211	

* Numerator indicates the mortality and the denominator the total number of mice on experiment.

three out of four of the groups. Mice receiving the lower level of the drug one day post-infection showed the highest mortality (81.8 per cent). The other three groups similarily treated after the infecting dose, showed increases in mortality ranging from two to eight per cent over the control group. No deaths were observed in the non-infected mice receiving drug alone.

DISCUSSION

Although there was some indication that mice receiving the drug prior to infection were in some degree protected as indicated by a lower mortality rate, the difference between these and the control group was not of statistical significance. Further, these experiments were conducted in an effort to evaluate the efficacy of Di-Paralene as an antifungal agent against established fungous infections. The results indicate that groups of mice receiving the drug following infection had a higher mortality rate than the control mice. Thus there appears to be some evidence that the drug had a potentiating effect upon these infections.

Roth, *et al.* (6) have established the acute toxicity levels of Di-Paralene by a number of routes and have evidence of the chronic toxic level when administered orally. We have, during the course of these experiments, established the fact that dosages of 10 mg./kg. to 25 mg./kg. when given daily to mice by the subcutaneous route provoke no abnormal responses or findings. Histologic sections on control mice receiving 25 mg./kg. daily for 45 days showed no significant abnormalities.

Cultures on two out of five mice which developed encephalitis during the course of the experiments showed the presence of *C. albicans* in the brain. Cultures were negative on the other three mice. As there was no correlation between the total dosage, and the development of encephalitis, it was felt that the drug was not incriminated. These five mice represent slightly less than one per cent of the total number of mice on experiment.

CONCLUSIONS

1. Di-Paralene at the dosage employed did not demonstrate therapeutic value, but on the contrary appears to have a potentiating effect upon systemic infections in mice with *C. albicans* and *C. neoformans.*

2. Mice are able to tolerate dosages up to 25 mg./kg. of Di-Paralene daily for 45 days without any evidence of acute or cumulative toxicity.

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