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In Vivo Studies with Ambruticin in Murine Histoplasmosis

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Ambruticin (W7783) was evaluated in vivo in mice subacutely or nonlethally infected with *Histoplasma capsulatum*. Results were compared with those obtained with amphotericin B, the drug of choice in human histoplasmosis. In one experiment, ambruticin was shown to be capable of curing infected animals as evidenced by totally negative liver and spleen cultures obtained when mice were sacrificed after 4 weeks of oral treatment with 150 mg of drug per kg per day. The 50% cure dose for ambruticin was between 75 and 150 mg/kg per day; the 50% cure dose for oral amphotericin B in this experiment was between 1.56 and 6.25 mg/kg per day. In a second experiment, both oral ambruticin (150 mg/kg per day) and oral amphotericin B (25 mg/kg per day) were again curative, but to a lesser degree than in the first experiment. Biological cures were obtained with both drugs after 3 and 4 weeks of treatment but not after 2 weeks.

Ambruticin (W7883) has been shown to be a relatively broad-spectrum antifungal agent active in vitro against a variety of filamentous and dimorphic fungal pathogens (4-6). It already has been shown to be active in vivo in mice experimentally infected with *Coccidioides immitis* and, moreover, capable both of protecting experimental animals against infection with this organism and of producing biological cures (1, 2). The studies to be reported here compared the in vivo efficacy of orally administered ambruticin with that of orally administered amphotericin B in mice experimentally infected with *Histoplasma capsulatum*.

MATERIALS AND METHODS

In vivo model. Two series of experiments were performed using a subacute, or nonlethal, in vivo model in mice infected with spore suspensions prepared from mycelial-phase cultures of a clinical isolate of *H. capsulatum* (Medical College of Virginia Mycology Culture Collection no. 18.33). Inocula were prepared by harvesting mature cultures with saline with subsequent filtration through gauze to remove particulate matter. Mice were infected by intravenous inoculation (lateral tail vein). Treatment was begun 3 days after infection. In the first experiment, the infecting challenge dose contained 2×10^4 colony-forming units; in the second experiment, it contained 2×10^5 colony-forming units.

Treatment. Infected and noninfected control animals were treated orally for 28 days. Ambruticin, diluted in saline, was administered orally by gastric intubation three times daily and amphotericin B, suspended in saline, was administered orally once daily. In the first experiment, total daily doses of ambruticin ranged from 19 to 150 mg/kg; daily doses of amphotericin B (Fungizone) ranged from 0.39 to 25 mg/kg. In the second experiment, ambruticin was administered three times daily in a total daily dose of 150 mg/kg, while amphotericin B was administered once daily in a single daily dose of 25 mg/kg. In each experiment, one group of infected animals was treated three times a day with a saline placebo identical in composition to the dosing vehicle used for both drugs. Treatment and infected or noninfected control groups consisted of 15 animals each in the first experiment and 10 animals each in the second experiment.

Cultural studies. In both experiments, cultural studies were performed on target organs of surviving animals at the end of the treatment period. In these studies, spleens and livers were removed from sacrificed survivors, placed in 3- to 4-ml volumes of saline contained in plastic bags, and squashed to produce suspensions which were then plated on Mycosel agar (BBL) with added chloramphenicol. Approximately 1 ml, or 25% of each organ suspension, was plated. The inoculated plates were incubated at 28°C until sufficient growth was present to permit accurate mycological identification. Growth was scored on the basis of + (≤10 colonies), ++ (10 to 100 colonies), and +++ to ++++ (greater than 100 colonies, or confluent growth with few or no discrete colonies). Identifications for at least one-third of all positive plates from each group of animals were confirmed by microscopic examination.

In the second experiment, 10 treated, infected mice from each of the two treatment regimen groups as well as the group of placebo-treated mice were sacrificed at the end of each week of treatment. One group of 10 infected but untreated mice was sacrificed on the first day of treatment. Livers and spleens were removed from these animals and processed as above for cultural studies.

RESULTS

Little or no mortality was seen in the first experiment with mice subacutely infected with *H. capsulatum*. No more than two deaths occurred in any treatment or control group, and no comparison could be made between ambruticin and amphotericin B or placebo regarding their relative protective efficacies. However, clear evidence was obtained regarding the ability of the two drugs to produce biological cures in infected mice (Table 1). Of 15 mice treated with 150 mg of ambruticin per kg per day, 13 survived; livers and spleens from 12 survivors were cultured. Biological cures were seen in 10 of these mice, as both liver and spleen cultures were negative on culture; livers from 2 additional mice were negative, but production of biological cures was uncertain because of contaminated spleen cultures. Complete biological cures were not seen at lower dosages of ambruticin, although spleens (but not livers) from four mice receiving 75 mg of ambruticin per kg per day were negative for *H. capsulatum*.

All mice receiving 6.25 or 25 mg of amphotericin B per kg per day were negative on culture of target organs for *H. capsulatum*, as were approximately one-third of mice receiving 1.56 mg/kg per day. Cures were not seen in mice receiving 0.39 mg/kg per day, as all organs were grossly positive, ++ to +++, for *H. capsulatum*. The approximate 50% cure dose for amphotericin B in this first experiment was between 1.56 and 6.25 mg/kg per day; for ambruticin, it was between 75 and 150 mg/kg per day.

The second experiment was designed to determine "time-to-cure" aspects of treatment with ambruticin in mice subacutely infected with *H. capsulatum* (Table 2). Thus, treatment with both drugs consisted only of the maximum daily dose of 150 mg/kg for ambruticin and 25 mg/kg for amphotericin B. All 10 mice sacrificed at the onset of the treatment phase, but without treatment, were grossly positive, +++ to ++++, on culture of spleen and liver for *H. capsulatum*. Nine of the 10 mice yielded spleen culture scores of 4-plus, while 6 had similar liver culture scores (data not shown). In contrast, whereas all treated mice sacrificed at the end of 2 weeks of treatment had spleens or livers positive for *H. cpasulatum*, average culture scores were reduced both for spleens and livers from mice treated with amphotericin B, to an average level of ++, and for livers from mice treated with ambruticin, to an average level of ++. The average culture score at this time for untreated animals was 4-plus for both liver and spleen.

After 3 weeks of treatment, only 1 of 10 mice treated with amphotericin B had a spleen positive for *H. capsulatum*. This was a single positive isolation from an animal which was negative on culture of liver. Nine of the animals yielded only + or ++ positive liver cultures. Three mice treated with ambruticin were negative on culture of spleens, and another three were negative on culture of livers. The remaining cultures of spleen were all + or ++ positive, while the remaining liver cultures were + or +++. One of the placebo-treated animals yielded a negative spleen culture; the remaining spleen cultures

TABLE 1. *Results of organ cultures from mice infected with H. capsulatum and orally treated for 28 days with ambruticin or amphotericin B*

Drug and dose (mg/kg)	Spleen cultures			Liver cultures		
	Negative	Positive[a]	Avg plate score[b]	Negative	Positive[a]	Avg plate score[b]
Ambruticin[c]						
150	10	0	0	12	0	0
75	4	8	+++	0	11	+++
38	0	11	+++	0	14	+++
19	0	10	+++	0	14	+++
Nontreated	0	13	+++	1	14	+++
Amphotericin B[d]						
25	14	0	0	14	0	0
6.25	14	0	0	14	0	0
1.56	7	6	+	3	10	+++
0.39	1	14	++	0	15	+++

[a] Number of cultures positive for *H. capsulatum*, with a minimum of one-third being confirmed microscopically.

[b] Average score of plates positive for *H. capsulatum*, on basis of 0 to +++ (greater than 100 colonies).

[c] Total daily oral dose, given in three divided doses.

[d] Total daily oral dose, given once daily.

TABLE 2. *Results of organ cultures from mice infected with H. capsulatum and treated orally for up to 28 days with ambruticin or amphotericin B*

Drug (dose)	No. of organ cultures positive for *H. capsulatum* (avg plate score)[a]					
	Week 2		Week 3		Week 4	
	Spleen	Liver	Spleen	Liver	Spleen	Liver
Ambruticin (150 mg/kg per day)	9/9 (+++)	8/9 (+++)	4/7 (+)	4/7 (+++)	3/8 (+)	6/8 (++)
Amphotericin B (25 mg/kg per day)	10/10 (++)	10/10 (++)	1/10 (0)	9/10 (++)	0/9 (0)	3/9 (+)
Placebo	10/10 (+++)	10/10 (++++)	9/10 (++)	10/10 (+++)	8/8 (+++)	8/8 (++++)

[a] Average score of plates positive for *H. capsulatum*, on basis of 0 to +++ (greater than 100 colonies) or ++++ (confluent growth).

were scored as + to +++, while the corresponding liver cultures were scored as ++ to ++++.

Six of nine animals treated with amphotericin B for 4 weeks were scored as biological cures on the basis of paired negative spleen and liver cultures. The remaining three mice had negative spleen cultures, while cultures of livers were positive, yielding one or two colonies of *H. capsulatum* per organ. This is in contrast to results from the first experiment in which all animals treated with either 6.25 or 25 mg of amphotericin B per kg per day were culturally negative at the end of 4 weeks of treatment. A similar reduction in efficacy was observed with ambruticin. Two of eight survivor mice treated with ambruticin were scored as biological cures. Three more were negative on culture of spleen but positive on culture of liver, yielding from three to five colonies in two instances and with a score of ++ in the third. Only one animal treated with ambruticin yielded culture results comparable to those seen in the placebo-treated animals. All eight of the surviving placebo-treated animals yielded positive spleen and liver cultures; scores ranged from ++ to ++++ and were greater than those obtained with placebo-treated animals from the first experiment. These results, combined with the lessened efficacy observed with both drugs, suggest that the infection was more severe in the second experiment.

While no attempt was made to make objective records of gross pathology in either experiment, certain correlations between culture results and organ pathology were noted. In the first experiment, little or no hepatomegaly or splenomegaly was noted in mice receiving the highest doses of ambruticin, and little was seen in mice receiving the next lower dose. Similar observations were noted in mice treated with amphotericin B. Similarly, whereas some organ enlargement was observed during the first 2 weeks of the second experiment in mice receiving both drugs, none was observed in mice sacrificed at the end of the experiment.

DISCUSSION

The results presented here show conclusively that orally administered ambruticin was effective in vivo both in reducing the number of viable cells of *H. capsulatum* in tissues of infected mice and in curing infected mice when administered in sufficient amounts. This activity, however, was not comparable to that of orally administered amphotericin B in similarly infected mice.

Data from the first experiment indicated that the 50% cure dose of ambruticin was in excess of 75 mg/kg per day. Blood levels determined in selected animals some 7 h after their last treatment with ambruticin indicated that those mice receiving the top two doses of ambruticin, 75 and 150 mg/kg per day, had ambruticin levels of between 6 and 16 μg/ml; the average level in mice receiving 150 mg/kg per day was 11.2 μg/ml. All other doses yielded no detectable blood levels (S. M. Ringel, personal communication). Historically, it is important to note that amphotericin B administered orally at a dose of 50 mg/kg per day was originally found by others to be curative in mice infected with *H. capsulatum* as well as capable of providing blood levels in treated animals in the range of 0.4 to 0.5 μg/ml (3).

Together, the high 50% cure dose of ambruticin in these experiments and the absence of detectable blood levels of ambruticin in mice receiving lower doses of the drug indicate that the optimal dose for this drug has yet to be determined. Apart from this problem of adequate dosage, one fact remains clear: ambruticin is capable of curing animals experimentally infected with *H. capsulatum*. This, together with similar data for *C. immitis* (1, 2), suggests that this drug may become an important and clini-

cally significant antifungal chemotherapeutic agent.

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LITERATURE CITED

1. **Levine, H. B., and S. M. Ringel.** 1977. Efficacy of W7783, a new oral antibiotic in experimental coccidioidomycosis, p. 319–322. *In* L. Ajello (ed.), Coccidioidomycosis: current clinical and diagnostic studies. Am. Col. Chest Physicians. Symposia Specialists Publications, Miami, Fla.
2. **Levine, H. B., S. M. Ringel, and J. M. Cobb.** 1978. Therapeutic properties of oral ambruticin (W7783) in experimental pulmonary coccidioidomycosis of mice. Chest **73**:202–206.
3. **Louria, D. B., N. Feder, and C. W. Emmons.** 1957. Amphotericin B in experimental histoplasmosis and cryptococcosis, p. 870. *In* H. Welsh and F. Marti-Ibanez (ed.), Antibiotics annual 1955–1956. Medical Encyclopedia, New York.
4. **Ringel, S. M.** 1978. In vitro and in vivo studies of ambruticin (W7783): new class of antifungal compounds. Antimicrob. Agents Chemother. **13**:762–769.
5. **Ringel, S. M., R. C. Greenough, S. Roemer, D. Connor, A. L. Gutt, B. Blair, G. Kanter, and M. von Strandtmann.** 1977. Ambruticin (W7783), a new antifungal antibiotic. J. Antibiot. **30**:371–375.
6. **Shadomy, S., D. M. Dixon, A. Espirel-Ingroff, G. E. Wagner, H. P. Yu, and H. J. Shadomy.** 1978. In vitro studies with ambruticin, a new antifungal antibiotic. Antimicrob. Agents Chemother. **14**:99–104.