Hamycin Treatment of Experimental Blastomycosis in Mice. (30567)

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Hamycin, a new antifungal antibiotic, has been reported to be effective in treatment of experimental fungal infections as well as in some systemic mycoses in man(4,5). A previous report from this laboratory documented the efficacy of the drug in treatment of experimental fungal infections over a limited range of doses(6). This report contains results obtained in experimental blastomycosis by expanding the range of doses to include minimal effective and toxic dose levels by the intraperitoneal route.

Since the chief advantage of this antibiotic over amphotericin B is its absorption when given orally(7,8), infected mice were treated orally to document the drug's efficacy *via* this route of administration.

Methods. Chemotherapeutic activity was tested by use of the experimental model previously reported(6,9).

Hamycin (Mycology Section Accession #B-2444) was obtained from Dr. M. J. Thirumalachar, Research Laboratories, Hindustan Antibiotics Ltd., Pimpri, near Poona, India. The hamycin used for intraperitoneal administration was prepared by suspending measured amounts of the drug in 0.1% sodium carboxymethyl cellulose in 0.85% saline. The mice treated by intraperitoneal injections were treated 5 days per week for a total of 20 treatments, and were then observed for 10 days prior to sacrifice. Treatment was started the third day after infection.

The hamycin for oral administration was prepared by mixing hamycin with powdered milk (Robbins) and adding weighed amounts of this mixture to 2-inch discs of white bread. The concentration of hamycin in the powder ranged from 0.125% to 5.0%.

The mice treated orally were allowed mouse laboratory chow (Country Best Foods) and water *ad libitum* in addition to the bread covered with hamycin-powdered milk mixture. Since the mice had free choice of food, an effort was made to quantitate the average amount of hamycin ingested per mouse per day. This was accomplished by weighing the medicated bread discs at time of feeding. The residual disc and bread crumbs remaining after 24 hours were weighed. The amount of drug ingested per mouse was then calculated by knowing the weight of bread disc prior to adding the drug, weight of drug-milk powder added, the dose of drug (as per cent of the hamycin-powdered milk mixture by weight), average weight loss of the bread discs due to dehydration, and number of mice remaining in each treatment group. Because of the number of variables, the figures for drug intake are only approximations.

The mice were treated orally for 19 consecutive days starting the third day after infection, and were observed for 18 days after the end of the treatment period prior to sacrifice.

The observation periods of 10-18 days after the end of the treatment period were designed to allow relapse of infection to occur if the infecting organism had not been eradicated, and to allow elimination of drug from tissues before cultures were made.

Results. The experimental blastomycosis produced in the untreated (vehicle control) mice was quite severe, average survival times being 8.8 to 8.9 days.

A. *Parenteral treatment study.* In spite of the severity of the infection, intraperitoneal treatment (Fig. 1) produced a chemotherapeutic effect in mice treated with the first 6 dose levels. The 3 high dose levels clearly delineate the drug toxicity with most mice dying after one to 3 injections of the drug.

The low dose (0.01 mg/mouse/day) produced a suppressive effect with only 2 deaths during the treatment period, and then a prompt exacerbation of disease when treatment was stopped at day 29. There were 6 deaths during the 10-day period following

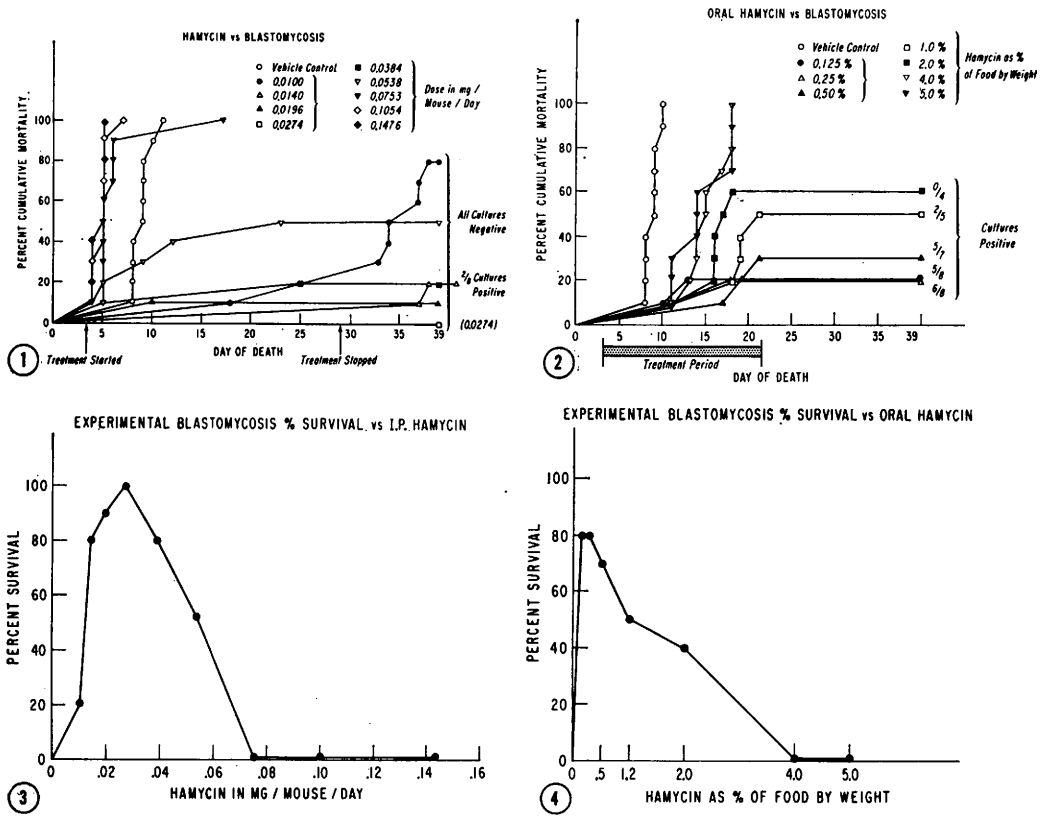

FIG. 1-4

cessation of treatment. Cultures from the 2 surviving mice were negative.

There was a similar exacerbation of infection when treatment was stopped in the mice receiving 0.014 mg/mouse/day, and 2 mice died just prior to time of sacrifice. Cultures from 2 of the mice surviving to be sacrificed were positive; those from the remaining 6 were negative.

The one mouse lost in the group treated with 0.0196 mg/mouse/day was accidentally killed on day 10. The remaining 9 mice survived to be sacrificed and all cultures taken at that time were negative.

All mice receiving 0.0274 mg/mouse/day survived to be sacrificed and all cultures were negative.

The next 2 doses (0.0384 and 0.0538 mg/mouse/day) approached the toxic level with 2 and 5 deaths, respectively, attributed to drug toxicity. Cultures from all mice in these 2 groups surviving to be sacrificed were negative. No mice receiving the 3 high doses (0.0753, 0.1054 and 0.1476 mg/mouse/day) survived to be sacrificed. Average duration of life in these 3 groups was 6.4, 4.9 and 4.6 days, respectively.

B. *Oral treatment study.* In the oral treatment studies, all of the doses used produced some chemotherapeutic effect. Average survival time of untreated mice was 8.8 days.

These mice (Fig. 2) were treated with 7 dose levels for 19 consecutive days, then observed for an additional 18 days without treatment. The conversion of dose in per cent of mixture to the calculated average dose per mouse is found in Table I.

The 4.0% and 5.0% doses were toxic, although partially protective so that the average survival times were 15.3 and 14.7 days, respectively. There was a progressive increase in mortality with increase in dose in the 5 lower doses also. There were survivors at each of these doses, however, and cultures taken at time of sacrifice revealed a decrease in percentage of positive cultures with in-

TABLE I. Oral Administration of Hamycin.

% Hamycin in powdered milk	Calculated dose in mg/mouse/day
.125	1.1
.25	2.1
.50	4.2
1.00	7.3
2.00	10.4
4.00	16.3
5.00	16.5

crease in dose. At the 2.0% dose, all cultures were negative.

Discussion. The new polyene antifungal antibiotic, hamycin, was effective in treatment of experimental blastomycosis in the mouse. This was true when the drug was administered orally or intraperitoneally. The per cent survival in relation to dose administered *via* the 2 routes is shown in Fig. 3 and 4. The results of the study utilizing intraperitoneal treatment (Fig. 3) clearly show the narrow range between minimal effective and toxic dose levels. There is, however, an optimum dose range which is both curative and nontoxic.

The study utilizing oral treatment did not delineate a dose which was curative and nontoxic. The toxic dose was delineated (Fig. 4).

It is probable that the duration of treatment was too short to arrive at a curative dose in the oral treatment study. The calculated doses used were much higher than those reported by Thirumalachar to be curative and nontoxic in experimental blastomycosis(2) and cryptococcosis(1) in mice. The studies are not comparable, however, because of the chronicity of the infection produced in the previous studies in India in contrast to the acute infection produced in the present study.

Colloidal hamycin(3) administered orally in smaller doses over a longer period was effective in treatment of experimental cryptococcosis in mice at doses which were nontoxic. Amphotericin B in both the crystalline (10) and colloidal(11) form given orally was effective in treatment of experimental mycoses in the mouse. Subsequent trials of both forms of amphotericin B given orally to patients with systemic mycoses were disappointing(12-18), however.

Recent reports of successful hamycin treatment of human disease caused by *Candida albicans*(4,5) and *Aspergillus niger*(4) are encouraging, as was the report of arrest of epizootic lymphangitis in horses(19) with oral hamycin treatment. On the basis of the efficacy demonstrated in these reports, clinical trials, using oral hamycin in treatment of proven systemic North American Blastomycosis in man, are in progress.

Summary. Hamycin, a new polyene antibiotic was effective against experimental blastomycosis in mice when injected intraperitoneally at levels between 0.01 and 0.0538 mg/mouse/day. The lowel level was only partially effective and the upper level was toxic. Hamycin was partially effective when administered orally as 0.125—5.0% by weight of food but 4.0% and 5.0% food mixtures were toxic.

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Ependymomas Produced in Syrian Hamsters by Adenovirus 7, Strain E46 ("Hybrid" of Adenovirus 7 and SV40). (30568)

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Several human adenoviruses have been shown to grow poorly in monkey kidney cell cultures in the absence of simian virus 40 (SV40), and marked enhancement of adenovirus growth has occurred when SV40 was added to the cultures(1). After the discovery of SV40(2), it was found that the "monkey kidney cell adapted" strains of human adenoviruses used for vaccine production were mixtures of adenovirus and SV40. To obtain a strain of adenovirus 7 free of SV40, vaccine strain LL was passaged 3 times in African green monkey kidney (AGMK) cell cultures in the presence of high titered SV40 antiserum. After the 3 passages, infectious SV40 could no longer be detected in the preparations although the virus grew well in AGMK cell cultures. When this strain (designated E46, substrain of strain LL) was inoculated subcutaneously in newborn hamsters, it produced subcutaneous tumors which contained the neoantigen of SV40(3). Evidence has been presented by Huebner and his associates(3), Rowe and Baum(4), and Rapp and his associates(5) that the portion of the SV40 genome which codes for production of the SV40 neoantigen is incorporated in some way within the capsid of the adenovirus 7 virion.

The subcutaneous tumors produced by E46 were described as resembling SV40 tumors grossly and microscopically although some tumors "also showed islands of epithelioid cells similar to those regarded as characteristic for adenovirus 12 tumors"(3). Since both SV40 and adenovirus 7 produce subcutaneous tumors after subcutaneous inoculation in newborn hamsters(6,7) and since these tumors do have certain histologic similarities as well as differences, it is difficult to be certain of the role played by the portion of the SV40 genome in the E46 strain of adenovirus 7 in tumor induction. When SV40 is inoculated intracerebrally in newborn hamsters, however, it produces well differentiated papillary ependymomas(8) which are readily distinguished from other tumors which have been produced by any known strains of oncogenic adenoviruses(7). This suggested to us that the inoculation of E46 intracerebrally into newborn hamsters might determine whether the portion of the SV40 genome believed to be within the adenovirus capsid was capable of inducing the characteristic SV40 ependymoma.

Materials and methods. Animals: Pregnant Syrian hamsters (*Cricetus auratus*) were obtained from the Animal Production Section of Nat. Inst. of Health.

Viruses: The Gomen strain of adenovirus 7, obtained from the American Type Culture Collection, was passed in Hep-2 cell cultures. Strain E46 was obtained from Dr. Wallace P. Rowe. Two pools of E46 were prepared in our laboratory. One, designated E46H1, was the first passage of the original material in human embryonic kidney (HEK) cell cul-

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