Evaluation of Systemic Antifungal Agents in X-Irradiated Mice

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The effect of X irradiation on the survival time of animals experimentally infected with pathogenic fungi was studied, and the activity of antifungal agents in preirradiated hosts was evaluated. A 24-hr preinfection dose of X irradiation decreased the survival time of mice infected with Cryptococcus neoformans and Histoplasma capsulatum to a greater extent than Candida albicans or Blastomyces dermatitidis infections. Exposure to 400 r caused a significant reduction in the variation (S²) survival time of C. albicans or H. capsulatum mouse infections. A single 100-mg/kg dose of 5-fluorocytosine or amphotericin B administered within 24 hr postinfection significantly extended the survival time of mice infected with C. albicans. Delayed treatment with amphotericin B was effective against C. neoformans infections. Four 50-mg/kg doses of 5-fluorocytosine were more effective than a single 200-mg/kg dose against C. neoformans infections. A single dose of amphotericin B provided significant protection when administered 48 hr postinfection against B. dermatitidis in preirradiated mice. A single dose of saramycetin 48 hr postinfection was highly effective against H. capsulatum mouse infections. A 100-mg/kg dose of amphotericin B was only effective against this fungal pathogen when administered within 8 hr postinfection. In vivo activity of the antifungal agents studied was detected within 8 to 14 days. The relative in vivo activity of several antifungal agents indicated the importance of considering their individual pharmacological properties for optimum effectiveness. The experimental model used in this study should be useful for the detection and for the preclinical evaluation of new antifungal agents.

Agents such as endotoxin (9), some antibiotics (15, 19), immunosuppressants (6, 13), and X irradiation (14, 17) have been used to suppress host responses to enhance microbial infections. Several clinically useful antifungal agents were effective against experimental fungal infections in cortisone-conditioned animals (3, 18; E. Grunberg, E. Titsworth, and M. Bennett, Antimicrob. Ag. Chemother.—1962, p. 566–568). Torulopsis glabrata showed an increased proliferation in mice X-irradiated prior to infection (7). The modification of experimental hosts with X irradiation prior to infection with Candida albicans altered the distribution of mycotic lesions and aligned with experimental infection more closely with naturally occurring human septicemic candidiasis (8).

This study was done to show the effect of X irradiation on mice infected with certain fungi and to evaluate the activity of amphotericin B, 5-fluorocytosine, and saramycetin in such infections.

MATERIALS AND METHODS

Candida albicans A26 and Cryptococcus neoformans WS 34 were grown at 30 C for 48 hr and Blastomyces dermatitidis 6059 and Histoplasma capsulatum 26 at 37 C for 96 hr in yeast phase on Sabouraud agar as modified by Emmons (2). The isolates of pathogenic fungi showed in vitro susceptibility to the antifungal agents used in all in vivo studies. Mice (ICR of 18 to 20 g) were inoculated in the lateral tail vein with 0.1 ml of cell suspension standardized to optical density of 660 nm which was then compared to a curve based on plate counts (4). Mice were X-irradiated 24 hr preinfection, as described previously, by using a 250-kV Westinghouse X-Ray Unit (5). Mice were preirradiated with sublethal doses of 400 r in all studies pertaining to the chemotherapeutic activity of antifungal agents.

Antifungal agents were suspended in 0.125% methyl-cellulose (15 centipoises, Dow Chemical Company) for treating all experimental infections. Subcutaneous administration was used except where otherwise designated. All treatments were administered in 0.25-ml volumes to groups of six infected...
mice; uninfected control mice received the total antibiotic dose in a single 0.5-ml intraperitoneal injection. None of the antifungal agents caused death of uninfected control animals at the doses administered. Groups of 10 mice were used for irradiation studies and for virulence controls in the chemotherapy studies. Significant differences in the mean survival time between treated and untreated groups of mice in a given study were supported by t test. Standard error (SE) was used to denote variation within each individual experiment. The homogeneity of variances (S²) was determined by the F-test on the basis of three independent sets of observations (21). All statistical data were processed by an Ollivetti Underwood Programa 101 computer.

RESULTS

Although X irradiation had little effect on the average survival time of mice infected with B. dermatitidis, doses of as much as 500 r reduced the average survival time of mice infected with C. neoformans or with 3 × 10⁶ cells of C. albicans (Fig. 1). Mice infected with 10⁴ or 5 × 10⁴ cells of H. capsulatum showed at least a 57.2 and a 52.5% reduction, respectively, between 0 and 500 r. There was no homogeneity of variance of survival time for two of three independent observations of C. albicans infections and three independent observations of H. capsulatum infections. This indicated that 400 r significantly reduced variation in survival times (Table 1).

A single 100-mg/kg dose of 5-fluorocytosine or amphotericin B administered up to 24 hr postinfection significantly extended the average survival time of C. albicans-infected mice beyond that of untreated infected controls (Table 2). Amphotericin B and 5-fluorocytosine were highly effective against C. albicans infections; however, amphotericin B was more active than 5-fluorocytosine at doses of 12.5- or 25-mg/kg (Table 3).

A single 100-mg/kg dose of amphotericin B administered up to 24 hr postinfection significantly extended the average survival time of C. neoformans-infected mice, whereas a 48-hr postinfection dose was less effective (Table 4).

Doses of 5-fluorocytosine or amphotericin B administered to C. neoformans-infected mice at 1 hr preinfection and 1 hr postinfection did not show a significant advantage over doses administered at 0 and 2 hr postinfection (Table 5). A dose of 5-fluorocytosine (50 mg/kg) at 2 and 4 hr postinfection was more effective than the same dose administered at 0 and 2 hr (Table 6). A 50-mg/kg dose of 5-fluorocytosine at 0, 2, 5, and 24 hr postinfection was more effective against C. neoformans infections than a 100-mg/kg dose administered at 0 and 2 hr postinfection, and equally as effective as a 50-mg/kg dose at 0, 2, 5, and 24 hr.

A 100-mg/kg dose of amphotericin B administered as late as 48 hr postinfection significantly extended the average survival time of B. dermatitidis-infected mice (Table 7).

Orally administered amphotericin B showed greater activity than subcutaneously administered antibiotic against B. dermatitidis mouse infections (Table 8).

Fig. 1. Effect of a 24-hr preinfection dose of X irradiation on the survival time of mice experimentally infected with pathogenic fungi.
TABLE 1. Influence of X irradiation, 24 hr preinfection, on the variation of survival times

| Organism                  | Cells per mouse | Variation (S²) of survival times² | Homogeneity of variance |
|---------------------------|-----------------|-----------------------------------|-------------------------|
|                           |                 | Unirradiated | 400 R                   |                         |
| Blastomyces dermato-      | 10³             | 0.32        | 0.17                    | +                       |
| matitidis 6059            |                 | 1.15        | 0.50                    | +                       |
|                           |                 | 0.54        | 0.40                    | +                       |
| Cryptococcus neo-         | 1.5 × 10⁶       | 14.1        | 7.9                     | +                       |
| formans WS 34             |                 | 13.2        | 7.7                     | +                       |
|                           |                 | 12.7        | 17.6                    | +                       |
| Candida albicans A26      | 1.5 × 10⁶       | 5.8         | 0.45                    | -                       |
|                           |                 | 1.65        | 0.54                    | +                       |
|                           |                 | 10.60       | 0.50                    | -                       |
| Histoplasma capsulatum 26| 5 × 10⁶         | 35.40       | 0.27                    | -                       |
|                           |                 | 3.21        | 0.26                    | -                       |
|                           |                 | 56.90       | 0.50                    | -                       |

² Groups of 10 mice each were used for the unirradiated and irradiated observations. Three independent observations were made for each organism.

TABLE 2. Effect of postinfection time of treatment on the activity of 5-fluorocytosine or amphotericin B against Candida albicans A26 infections in preirradiated mice (8-day evaluation)²

| Postinfection treatment time (hr) | Avg survival time (days ± SE)² | Per cent extension of survival above untreated controls² |
|----------------------------------|--------------------------------|--------------------------------------------------------|
| 5-Fluorocytosine                 |                                |                                                        |
| 0                                | 7.2 ± 0.40                     | 108                                                   |
| +2                               | 8.0 ± 0.00                     | 150                                                   |
| +4                               | 7.7 ± 0.33                     | 141                                                   |
| +6                               | 7.7 ± 0.33                     | 141                                                   |
| +24                              | 7.5 ± 0.34                     | 134                                                   |
| Amphotericin B                   |                                |                                                        |
| 0                                | 8.0 ± 0.00                     | 150                                                   |
| +2                               | 7.0 ± 0.63                     | 119                                                   |
| +4                               | 7.5 ± 0.50                     | 134                                                   |
| +6                               | 8.0 ± 0.00                     | 150                                                   |
| +24                              | 7.7 ± 0.33                     | 141                                                   |

² Mice were given a single dose of 100 mg/kg.

² Untreated mice infected with 1.5 × 10⁶ cells had an average survival time of 3.2 ± 0.13 days.

² Significant (t test) at P ≤ 0.005.

A 100-mg/kg dose of amphotericin B administered as late as 8 hr postinfection showed significant activity against H. capsulatum infections, whereas a dose at 24 or 48 hr postinfection was ineffective (Table 9). A 6.25-mg/kg dose of saran-

TABLE 3. Activity of 5-fluorocytosine or amphotericin B against experimental Candida albicans A26 infections in preirradiated mice (8-day evaluation)

| Antifungal agent | Dose (mg/kg) | Avg survival time (days ± SE)² | Per cent extension of survival above untreated controls² |
|------------------|-------------|--------------------------------|--------------------------------------------------------|
| 5-Fluorocytosine | 12.5        | 5.2 ± 0.54                     | 86                                                    |
|                  | 25          | 5.8 ± 0.98                     | 107                                                   |
|                  | 50          | 6.5 ± 0.62                     | 132                                                   |
|                  | 100         | 7.5 ± 0.50                     | 168                                                   |
| Amphotericin B   | 12.5        | 7.8 ± 0.17                     | 179                                                   |
|                  | 25          | 7.8 ± 0.17                     | 179                                                   |
|                  | 50          | 7.0 ± 0.81                     | 150                                                   |
|                  | 100         | 7.7 ± 0.33                     | 171                                                   |

² Untreated mice infected with 1.5 × 10⁶ cells had an average survival time of 2.8 ± 0.25 days.

² All values were significant by t test at P ≤ 0.005.

TABLE 4. Influence of postinfection time of treatment on amphotericin B activity against Cryptococcus neoformans WS34 infections in preirradiated mice (13-day evaluation)²

| Postinfection treatment (hr) | Avg survival time (days ± SE)² | Per cent extension of survival above untreated controls² |
|-----------------------------|--------------------------------|--------------------------------------------------------|
| 0                           | 9.7 ± 0.84                     | 135                                                   |
| 4                           | 9.8 ± 0.67                     | 139                                                   |
| 24                          | 9.0 ± 0.60                     | 119                                                   |
| 48                          | 6.2 ± 2.2                      | 51                                                    |

² Each mouse received a single dose of 100 mg of amphotericin B per kg.

² Untreated mice infected with 2.0 × 10⁴ cells had an average survival time of 4.0 ± 0.6 days.

² Significant (t test) at P ≤ 0.025 level.

A 100-mg/kg dose of amphotericin B administered as late as 8 hr postinfection showed significant activity against H. capsulatum infections, whereas a dose at 24 or 48 hr postinfection was ineffective (Table 9). A 6.25-mg/kg dose of saran-

mycetin administered 8, 24, or 48 hr postinfection showed significant activity against H. capsulatum infections; a 3.12-mg/kg dose was less effective at 48 hr than at 8 or 24 hr postinfection (Table 10). Saramycetin administered at 3.12 to 12.5 mg/kg was highly effective against H. capsulatum infections, whereas amphotericin B administered at 2 and 4 hr postinfection was only effective at doses of 25 mg/kg or greater (Table 11).
expression of total leukocytes, (ii) a reduction in red blood cell and plasma volumes, and (iii) a higher albumin to globulin ratio of serum proteins attributed to decreases in alpha- and beta-globulins (5). The present study showed that preinfection exposure to X irradiation resulted in reduction of the average survival time of mice infected with several pathogenic fungi. Furthermore, preinfection exposure to 400 r significantly reduced variation (S2) of survival time of H. capsulatum and C. albicans mouse infections. A reduction in variation of survival time can relate directly to an increase in sensitivity of this system. Accordingly, compounds with low in vivo activity should be detected. These active compounds may show greater activity upon additional purification or structural modification.

In spite of differences in the average survival times for different experiments, each antifungal agent used in this study showed significant activity against a given fungal infection. An analogous
Table 9. Influence of postinfection time of treatment on the activity of amphotericin B against Histoplasma capsulatum 26 infections in preirradiated mice (10-day evaluation)

| Postinfection treatment time (hr) for amphotericin B (100 mg/kg) | Avg survival time (days ± SE)a | Per cent extension of survival above untreated controls |
|---------------------------------------------------------------|-------------------------------|------------------------------------------------------|
| 0                                                             | 6.7 ± 1.1                     | 153b                                                 |
| 2                                                             | 7.8 ± 1.4                     | 201b                                                 |
| 4                                                             | 8.2 ± 1.7                     | 214b                                                 |
| 6                                                             | 8.8 ± 1.5                     | 239b                                                 |
| 8                                                             | 8.3 ± 1.7                     | 221b                                                 |
| 24                                                            | 2.3 ± 0.21                    | 0                                                    |
| 48                                                            | 2.0 ± 0.25                    | 0                                                    |

a Untreated mice infected with 5.0 × 10⁶ cells had an average survival time of 2.6 ± 0.34 days.

b Significant (t test) at P ≤ 0.025 level.

Table 10. Influence of postinfection time of treatment on the activity of saramycetin against Histoplasma capsulatum 26 infections in preirradiated mice (13-day evaluation)

| Postinfection treatment time (hr) | Saramycetin (mg/kg) | Avg survival time (days ± SE)a | Per cent extension of survival above untreated controlsb |
|-----------------------------------|---------------------|-------------------------------|------------------------------------------------------|
| 8                                 | 3.12                | 9.8 ± 0.79                    | 56                                                   |
| 6.25                              | 12.5 ± 0.50         | 100                            |                                                       |
| 3.12                              | 9.5 ± 1.05          | 52                             |                                                       |
| 6.25                              | 13.0 ± 0.00         | 108                            |                                                       |
| 8                                 | 8.1 ± 0.48          | 29                             |                                                       |
| 6.25                              | 12.5 ± 0.50         | 100                            |                                                       |

a Untreated mice infected with 5.0 × 10⁶ cells had an average survival time of 6.2 ± 0.11 days.

b Significant (t test) for all values at P ≤ 0.025 level.

The effects of preirradiation on H. capsulatum infections may have been particularly dramatic since this fungus is an intracellular parasite of the radiosensitive reticuloendothelial system (22). The absence of uniform responses by X-irradiated mice to different infecting fungi suggests individuality of each experimental mycotic infection. The advantages of lowering host responses by exposure to nonlethal doses of X irradiation as compared to administering agents such as cortisone are the following: (i) a large number of mice can be X-irradiated rapidly with a minimum of trauma, (ii) a dose of X rays is a highly reproducible homogenous experimental parameter, and (iii) the possibility of direct chemical interactions with test compounds is eliminated.

Under the experimental conditions in this study, a single 100-mg/kg dose of amphotericin B administered within at least 24 hr postinfection was effective against C. albicans, C. neoformans, and B. dermatitidis; administration after 8 hr postinfection was ineffective against H. capsulatum. A somewhat lower dose of saramycetin was effective against H. capsulatum when administered 48 hr postinfection. The observation that a 200-mg/kg dose of 5-fluorocytosine in four divided doses is more effective than two doses against C. neoformans further suggests the need for individualizing the treatment schedule of an antifungal agent for each experimental mycosis.

Information concerning such pharmacological properties as duration and distribution of an antifungal activity in animal tissues is important for scheduling an optimum treatment regimen. For example, the half-life of 5-fluorocytosine is 4 to 8 hr, whereas amphotericin B activity in blood was detected after administration for at least 7 days and declined only 40% from the peak level of activity in 4 days (10, 12). The differences in the pharmacological properties of these two antifungal agents could partially account for the lower activity of 5-fluorocytosine compared with equivalent 12.5-mg/kg doses of amphotericin B.
against \textit{C. albicans}. Interestingly, repeated lower than normally recommended doses of amphotericin B maintained adequate human serum levels of antibiotic activity and were highly effective with less renal toxicity (D. J. Drutz, A. Spickard, and M. G. Koenig, Antimicrob. Ag. Chemother. —1966, p. 202–207).

Amphotericin B, hamycin, or saramycetin were shown previously to be effective against experimental fungal infections by daily administration for 28 to 30 days (1, 6, 11, 24; T. W. Williams Jr., J. E. Bennett, and C. W. Emmons, Antimicrob. Ag. Chemother.—1963, p. 737–741). A single dose 15 min postinfection or daily administration of 5-fluorocytosine for 21 days was effective against candidiasis in mice (16; Grunberg et al., Antimicrob. Ag. Chemother.—1962, p. 566–568). In the present study, with pre-irradiated animals, antifungal agents showed significant activity with a single dose or a short-term multiple-dose regimen within 8 to 14 days. This experimental model system provides a relatively rapid evaluation of materials for in vivo antifungal activity.

Unfortunately, a direct correlation between in vitro susceptibility and therapy of experimental animal infections with clinical efficacy is not always possible. This disparity has been attributed to differences in host responses and pathogenesis (20). Nevertheless, the high in vivo activity exhibited by clinically useful antifungal agents demonstrates that the experimental model used in this study should be useful for detecting and evaluating new chemotherapeutic agents for systemic fungal infections.

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