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BRIEF COMMUNICATION

FLUCYTOSINE + FLUCONAZOLE ASSOCIATION IN THE TREATMENT OF A MURINE EXPERIMENTAL MODEL OF CRYPTOCOCCOSIS

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SUMMARY

The efficacy of flucytosine (5-FC) and fluconazole (FLU) association in the treatment of a murine experimental model of cryptococcosis, was evaluated.

Seven groups of 10 Balb C mice each, were intraperitoneally inoculated with 10^7 cells of *Cryptococcus neoformans*. Six groups were allocated to receive 5-FC (300 mg/kg) and FLU (16 mg/kg), either combined and individually, by daily gavage beginning 5 days after the infection, for 2 and 4 weeks. One group received distilled water and was used as control.

The evaluation of treatments was based on: survival time; macroscopic examination of brain, lungs, liver and spleen at autopsy; presence of capsulated yeasts in microscopic examination of wet preparations of these organs and cultures of brain homogenate.

5-FC and FLU, individually or combined, significantly prolonged the survival time of the treated animals with respect to the control group ($p<0.01$). Animals treated for 4 weeks survived significantly longer than those treated for 2 weeks ($p<0.01$). No significant differences between the animals treated with 5-FC and FLU combined or separately were observed in the survival time and morphological parameters.

The association of 5-FC and FLU does not seem to be more effective than 5-FC or FLU alone, in the treatment of this experimental model of cryptococcosis.

KEYWORDS: Cryptococcosis; Experimental cryptococcosis; Antifungal drugs.

INTRODUCTION

Cryptococcosis is a systemic mycosis observed in immunocompromised hosts. In AIDS patients cryptococcosis is currently an important life-threatening mycosis [3-4, 9]. The combination of amphotericin B (AMB) and 5-FC is the treatment of choice; however, this treatment often produces side effects, it is also associated with high rate of relapses and must be administered only to hospitalized patients. The use of this association in AIDS patients is controversial due to its poor tolerance [3-5, 8-9].

On the other hand new azolic are currently available for the treatment of cryptococcosis: itraconazole (ITRA) and FLU. ITRA does not have a good passage across the blood-brain barrier; on the contrary FLU achieves therapeutic levels in cerebrospinal fluid and is avalaible for intravenous and oral administration [4, 5, 8-9].

The association of antifungal drugs is now being investigated as a possible alternative therapy to different fungal infections, including cryptococcosis [7, 10-11].

The aim of this study is to evaluate the efficacy of

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5-FC + FLU in a murine experimental model of cryptococcosis.

MATERIALS AND METHODS

1. - Inoculated animals

Seventy male Balb/C mice, weighing approximately 25 g, bred in the National Academy of Medicine, were employed. They were housed in 7 groups of 10 animals each; 6 groups were employed for treatments (3 groups for 2 weeks and 3 for 4 weeks) and 1 group was used as control. All animals received food and water *ad libitum*.

2. - Inoculation technique

The inoculum was performed with the Rivas strain of *Cryptococcus neoformans var. neoformans* suspended in sterile isotonic saline [2]. Animals were inoculated by intraperitoneal route (i.p.) with 10^7 cells.

3. - Drugs and schemes of treatments

The treatments were started 5 days after infection. The drugs were dissolved in distilled water and administered for 2 or 4 weeks by gavage at a daily dose of 300 mg/kg/day for 5-FC and 16 mg/kg/day for FLU, either as single-drug regimen or associated. The control group received distilled water by gavage.

4. - Treatment evaluations

The animals were left at their spontaneous evolution, after the experimental inoculation. At death all animals were autopsied and the following parameters were recorded:

a) survival time;
b) gross macroscopic aspect of the brain (presence of softening), lungs (presence of nodules), liver and spleen (presence of hepatosplenomegaly);
c) detection of capsulated yeasts in wet preparations

TABLE 1

Treatments of experimental murine cryptococcosis with flucytosine (5-FC) and fluconazole (FLU) alone or associated. Results of macroscopy, microscopy, survival, time and massive seeding of brain

Group	Number of animals	Macroscopy positive for			Microscopy positive				Survival time (average in days)	Massive seeding positive of Brain
		HSM	LN	SB	Li	Sp	Lu	Br		
Control	10	10	10	10	10	10	10	10	22.8 (16-30) ±2.41	10
Animals treated during 2 weeks										
FLU + 5-FC	10	10	10	10	10	10	10	10	35.1 (32-40) ±2.37	10
FLU	10	10	10	10	10	10	10	10	34.9 (30-44) ±4.25	10
5-FC	10	10	10	10	10	10	10	10	35.5 (32-40) ±2.41	10
Animals treated during 4 weeks										
FLU + 5-FC	10	10	10	10	10	10	10	10	48.6 (38-58) ±6.89	10
FLU	10	10	10	10	10	10	10	10	50.5 (46-56) ±3.97	10
5-FC	10	10	10	10	10	10	10	10	47.1 (40.56) ±5.08	10

Reference:
Lu: lung; Li: liver; Br: brain; Sp: spleen; LN: lung nodules; SL: Soltening lesions in CNS; HSM: hepatosplenomegaly

of brain, lung, liver and spleen, by contrast phase microscopy;

d) and development of *C. neoformans* in massive seeding of brain homogenate (100 mg/ml) in Sabouraud honey agar, incubated at 37°C, for 1 week.

5. - Statistics

The Mann-Whitney rank sum test was applied to analyze survival time.

RESULTS

As previously reported [2], in this murine experimental model, control mice die about 3 weeks after i. p. infection with 10^7 cell of *C. neoformans*.

The results obtained are summarized in the Table 1. 5-FC and FLU, individually or combined, prolonged significantly the survival time of the animals when compared to the control group ($p<0.01$). No significant differences between the animals treated with 5-FC and FLU, combined or separately, were observed in any of the studied parameters. All mice presented macroscopic and microscopic changes due to *C. neoformans*, with the same extention. Duration of treatments was an important variable, since 4 weeks resulted in longer survival than 2 weeks ($p<0.01$).

DISCUSSION AND CONCLUSIONS

Since no therapeutic schemes are active enough to cure cryptococcosis in AIDS patients, a maintenance treatment is indefinitely required [3-5, 9].

The association of different antifungal drugs is an interesting proposal for the treatment of cryptococcosis, considering that the regular therapy is unsuccessful. AMB plus 5-FC was the first combined treatment for this mycosis and other associations are being currently investigated [9-11].

POLAK observed that the association of 5-FC and azolic compounds has no antagonic effects and may be even sinergistic in experimental models of murine cryptococcosis, aspergillosis and candidiasis [10].

Using 5-FC + FLU ALLENDOERFER et al. [1] significantly delayed the mortality and observed a lower amount of *C. neoformans* in brain tissue of treated animals with respect to the control and single drug regimens [1]. They employed athymic Balb/C mice (nu/nu) intracerebrally inoculated with 150-300 CFU of *C. neoformans* and treated 24 hours post-infection for 10-14 days with FLU (1 to 15 mg/kg/day) and 5FC (60 to 120 mg/kg every 8 hours) in different combinations and single-drug regimens.

HITCHCOCK found that 5-FC (50 at 200 mg/kg) + FLU (1,25 at 10 mg/kg), administered for 7 to 10 days by gavage had an at least additive effect in mice intracerebrally inoculated with 500 CFU of *C. neoformans*. They observed a reduction in brain levels of *Cryptococcus neoformans* with several combinations of 5-FC and FLU with respect to either drug alone [6].

The reason for the difference between ALLENDOERFER and HITCHCOCK'S results and ours is unclear. It may be related to the different doses and duration of treatments, experimental infection route, animals employed and size of the inocula.

JONES et al. [7] treated successfully cryptococcosis in 15 AIDS patients with cryptococcosis with 5-FC (150 mg/kg/day) + FLU (400 mg/day) and SCHEVEN et al. [11] obtained similar results in the treatment of a *Candida albicans* sepsis with 5-FC (10 g/day) + FLU (600 mg/day). However the authors did not include control groups treated with both drugs separately, in their studies.

In a previous work we demonstrated the effectiveness of different antifungal treatments in a murine experimental model of cryptococcosis [2]. In the present investigation 5-FC + FLU did not show better results than those produced by each drug when they were administered separately, using the same experimental model.

In the future, other associations of antifungal drugs may be assayed in this experimental mice model of cryptococcosis, with the aim of finding out new therapeutical schedules.

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