Lack of In Vitro Antimicrosporidian Activity of Thalidomide

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Thalidomide was evaluated for its in vitro activity against Encephalitozoon species by using the MRC-5 cell system. A cytotoxic effect was observed for concentrations of 10^1 μg/ml (P < 10^-5) and 5 μg/ml (P < 10^-5).

Thalidomide did not significantly inhibit the growth of any of the microsporidia under study. These data suggest that thalidomide is not an etiologic treatment in microsporidial enteritis.

Microsporidia are spore-forming, obligate intracellular microorganisms known to parasite almost every group of animals (23). In humans, Enterocytozoon bieneusi, Encephalitozoon intestinalis, and Encephalitozoon cuniculi are emerging pathogens increasingly recognized as major causes of diarrhea, weight loss, and wasting in human immunodeficiency virus (HIV)-infected patients (5) and in non-HIV-infected patients (2, 11, 20, 22). Enteric infection is localized to the small intestine and is associated with villus damage and functional alterations (14). E. bieneusi infection is limited to the intestine, whereas Encephalitozoon species are also responsible for antimicrobial-resistant, life-threatening systemic infections (5, 7, 10).

Thalidomide, first developed as a sedative in the late 1950s, was withdrawn from widespread use because of teratogenicity (3). However, recently, thalidomide was approved for the treatment of leprosy (4). It has been shown to prevent HIV replication in monocytes in vitro (9) but has no known antimicrobial activity. It has also been successfully used as a treatment for microsporidial enteritis in HIV-infected persons (18), with improvement in clinical symptoms and histological parameters (16, 18). Microsporidial alterations were noted at all stages of the life cycle (18). These observations suggested a direct antimicrosporidian effect of thalidomide, but no experimental model has been developed to assess this hypothesis. We therefore tested the potential antimicrosporidian activity of thalidomide against E. intestinalis, E. cuniculi, and Encephalitozoon hellem using a cell culture system.

Thalidomide (LAPHAL, Paris, France) was dissolved in sterile dimethyl sulfoxide (DMSO) and the optical density (OD) at 492 nm (OD_{492}) of treated cells was 1 ml of 1% decomplemented fetal calf serum culture medium, with or without thalidomide, was added and the plate was incubated at 37°C in a 5% CO₂ incubator for 6 days. For each plate, line 1 was a positive control without drug, and nine serial dilutions of thalidomide from 10 to 10^{-5} μg/ml were tested in lines 2 to 6 of the plate. After incubation, fixed cells were stained by quick-hot gram-chromotrope staining, and coverslips were mounted on microscope slides and observed with a light microscope using a 100× oil immersion lens objective. The mean and standard deviation of the mean number of microsporidia per field were determined after 20 fields had been observed. The percentage of microsporidian growth inhibition was calculated as [1 - (mean number of infected cells in replicate cultures with thalidomide/mean number of infected cells in control cultures)] × 100 (± standard error).

Analysis of variance was used to compare the means and standard deviations of optical density in the toxicity test and percentages of growth inhibition for each microsporidian species and for each concentration of thalidomide.

A toxic effect was observed for thalidomide concentrations of 5 to 10 μg/ml (P < 10^-5) (Table 1). DMSO at a final concentration of 10^{-3} μg/ml had no toxic effect on MRC-5 cells. The percentage of growth inhibition varied from 0 to 8.2% for E. intestinalis, from 0 to 5.6% for E. cuniculi, and from 0 to 8.4% for E. hellem, with thalidomide concentrations of 10^{-1}, 5, 10^0, 10^{-1}, 10^{-2}, 10^1, 10^{-2}, and 10^{-5} μg/ml (Table 1). Statistical analysis indicated that no thalidomide concentration significantly reduced the growth of any of the three Encephalitozoon species under investigation. We did not observe morphological modifications in any of the three species under investigation.

Thalidomide was reported to relieve the clinical symptoms of microsporidian enteritis in HIV-1-infected patients as assessed by lower stool frequency and body weight gain (16, 18). Clinical improvement correlated with the normalization of the villus height/crypt depth ratio, along with morphological alterations of microsporidia (16). Electron microscopy analysis of pre- and post-thalidomide treatment intestinal biopsies disclosed no reduction in the microsporidian load but a significant increase in the number of ultrastructurally abnormal forms. These included membrane damage, vacuolated nuclei and cytoplasm, megaspores, and meganuclei for E. bieneusi. In the case of E. intestinalis, plasmodia and spores detected in the stool after treatment were disrupted (16). Data herein reported demonstrate that thalidomide had no direct antimicrosporidian effect resulting in such abnormalities and no significant microsporidian growth inhibition effect over the concentration range we studied. The model used in this study has been previously validated for antimicrosporidian screening of various drugs (1, 12). There is no pharmacokinetic study of thalidomide in HIV-1-infected patients, but a 5-μg/ml level in...
plasma was achieved in patients presenting with chronic graft-versus-host disease (21). One may assume that the concentration range we tested comprises expected levels in serum during the treatment of microsporidian enteritis. Elevated levels of fecal tumor necrosis factor alpha (TNF-α) were found in HIV-infected patients with microsporidial enteritis (17), and a marked, albeit nonsignificant, decrease of fecal TNF-α level from 17.9 to 8.9 U/ml was observed after thalidomide treatment (5). Percent inhibition = 1 – (mean number of infected cells in replicate cultures)/(mean number of infected cells in replicate controls) (100 ± standard error).

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