



Coccidioidomycosis

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Abstract 1: New serologic tests for Coccidioidomycosis

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At a university health center located within an area endemic for coccidioidomycosis, patients were tested for anticoccidioidal antibodies and circulating antigens using conventional antibody detection methods and new ELISA procedures. Of 233 patients who had symptoms compatible with coccidioidal infection, 26 had anticoccidioidal antibodies detected by conventional tests. ELISA detected antibodies in sera from 20 of these patients and also from an additional 25 patients. Patients with antibodies detected by either conventional or ELISA procedures were significantly more likely to have abnormal chest roentgenographs, elevated erythrocyte sedimentation rates, or the absence of upper respiratory symptoms than were patients without serologic evidence of coccidioidomycosis. Circulating antigen was found in serum from 35 patients, 33 of whom at that time had no detectable anticoccidioidal antibodies. Detectable antigen was noted frequently in sera obtained within the first month after the onset of symptoms and was less frequently detected later as progressively more patients developed antibody. These findings indicate that ELISA procedures for antibody and circulating antigen detection may increase the sensitivity threefold over standard serologic tests in the detection of early infections due to Coccidioides immitis.

Abstract 2: INHIBITION OF LABELED N-ACETYL GLUCOSAMINE UPTAKE OF COCCIDIOIDES IMMITIS BY HUMAN MONONUCLEAR CELLS

Neil M. Ampel, John Galgiani, George Bejarano and Rosemary Hayden

Tucson VAMC and the Arizona Health Sciences Center, the role of specific effector cell is in host immunity during coccidioidomycosis is not established. We have developed an assay which measures the uptake by *C. immitis* of the chitin precursor N-acetyl glucosamine (GlcNAc). In this study, we explore the effect of human peripheral blood mononuclear cells (MNL) on the uptake of GlcNAc by arthroconidia and spherules of *C. immitis*. MNLs from both immune and non-immune donors were able to inhibit GlcNAc incorporation in coccidioidal arthroconidia in a dose dependent manner. 12×10^6 MNL inhibited uptake by $69.8 \pm 8.8\%$ (N=5), a level equivalent to that seen with PMN. This Inhibitory activity dropped by $48.6 \pm 5.8\%$ (N=5) when monocytes were depleted from the mononuclear cell I fraction using a Sephadex G-10 column (P<0.005). Cell I fractions from skin-test negative and positive donors prepared using Sepracel I-MN and which contained >75% monocytes by non-specific esterase staining demonstrated significantly more inhibition of GlcNAc incorporation than fractions consisting of >90% lymphocytes (P<0.05). Further, adherent cells from the monocyte-rich fraction were significantly more Inhibitory than non-adherent cells is (P<0.05). GlcNAc uptake by spherules was inhibited by <25% by monocytes, a value similar to PMN. We conclude that human peripheral blood mononuclear cells are able to inhibit the incorporation of a chitin precursor of coccidioidal arthroconidia. This inhibition is independent of immune status of the donor and appears to reside specifically in the human peripheral blood monocyte.

Abstract 3: Itraconazole Therapy of Chronic Coccidioidal Meningitis

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Study objective: To assess the efficacy of orally administered IZ in the treatment of coccidioidal meningitis.

Design: Prospective, non-randomized open trial. Setting: Multicenter trial at one urban county hospital, a university referral center and referring institutions.

Patients: Ten patients (pts) with culture or serologic evidence of coccidioidal meningitis refractory to standard Rx. Pts receiving other systemic antifungal Rx were excluded. Intervention: IZ was administered orally at doses of 300q-400 mg/d for a median duration of 10 mo. Disease activity and drug efficacy were evaluated at initiation of Rx and at the most recent follow-up using a standardized scoring system. Measurements and Main Results: Eight of 10 pts are evaluable. Of 5 pts receiving IZ as sole Rx, 4 have responded. All 3 pts receiving intrathecal Amphotericin B have had that Rx discontinued and are without evidence of active disease in the absence of intrathecal Rx. Toxicity has been minimal; a single pt had mild nausea.

Conclusions: IZ shows impressive activity in this series of pts with refractory coccidioidal meningitis. Further evaluation of IZ in this and other fungal meningitides is in order.

Abstract 4: The Toxicity of Itraconazole in 189 Patients on Chronic Therapy

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Itraconazole was administered at doses of 50-400 mg/day to 189 pts with a variety of systemic mycoses for a median of 5 months. Adverse reactions possibly due to IZ were seen in 73 pts (39%); *mild* gastrointestinal reactions were most common. No fatal reactions have been noted and toxicity has rarely led to discontinuation of Rx. Chronic Rx with IZ appears well tolerated by the majority of pts.

Abstract 5: Fluconazole for Coccidioidomycosis

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We have used fluconazole in 74 patients with chronic pulmonary (42), skeletal (14), or soft Tissue (18) coccidioidal infections using daily doses of 50-100 mg (early study: 20 patients) and 200-400 mg (current study: 54 new patients & 5 relapsing after 100 mg). Efficacy was assessed every 3-4 mos by standard scoring of symptoms, appearance of lesions, serologic results, and fungal cultures with reduction in baseline abnormalities (any $<$ by 3-4 mos; $\geq 50\%$ $<$ by 6-8 mos) considered a response. Those not responding to 200 mg were offered 400 mg. With 50-100 mg, abnormalities improved 31 ± 7 and $36\pm 9\%$ (mean \pm sem) at 3 & 6 mos. By 8 months, 6 of 16 (38%) and overall 7 (44%) of evaluable patients responded. With 200-400 mg, median time on therapy is currently 8 mos with 93% of patients still receiving fluconazole. Results at 4 mos (n=31): $36\pm 5\%$ improvement, 25 patients continue on 200 mg, and 5 of 6 non-responders changed to 400 mg. At 8 mos (13 receiving 200 mg; 4 receiving 400 mg): $45\pm 6\%$ improvements, 10 patients (59%) are responders (8 on 200 mg and 2 on 400 mg), and 4 additional patients have begun 400 mg. A confirmed reversible drug reaction has stopped treatment in only one patient receiving 200 mg. 200-400 mg of fluconazole is well tolerated and may be effective.

Abstract 6: COCCIDIOIDOMYCOSIS IN SUBJECTS INFECTED WITH HIV A PROSPECTIVE STUDY

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Coccy has been reported to be a widely disseminated disease among HIV-infected individuals. We have initiated a prospective study of HIV-infected subjects living in an area endemic for coccy. To date, 73 subjects have been entered (median follow-up 6 mos). 4 subjects have active coccy. Analysis of the other 69 subjects reveals that 14 have evidence of prior coccy, 26 have no evidence of prior coccy and 23 are too immuno-suppressed to make a determination. Data is incomplete in 6. Hence 27% (18/67) of all subjects entered either have active coccy or prior evidence of infection. We have also retrospectively identified 12 additional cases of active coccy in Tucson not previously reported. Of the 16 total active cases, 6 had pulmonary disease (3 with reticulonodular pattern), 4 had meningitis, 3 had positive serologies only, 2 had positive mediastinal nodes and 1 had dissemination to the skin. 3 subjects have died all with reticulonodular pulmonary infection. 7 were still alive >1 yr after diagnosis of coccy. Treatments have included Amphotericin S, ketoconazole, fluconazole & itraconazole. We conclude that cocci is a major risk among HIV-infected individuals living in an endemic area. Its manifestations appear to be much more varied than previously reported.

Abstract 7: RISK OF *C. IMMITIS* MENINGITIS ASSOCIATED WITH PREGNANCY

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The incidence of *C. immitis* meningitis in pregnant females was determined from a retrospective chart review of all cases between January 1, 1982 and December 31, 1988, at Kern Medical Center Bakersfield, California. A total of four cases of *C. immitis* meningitis developing in the third trimester or post-partum were identified from a total of 68,706 births. This gave an overall incidence of 5.8 cases per 100,000 deliveries. Of these four cases, all presented with either a military or disseminated disease within either the third trimester or the post-partum period. They had a mean age of 22 years

	<u>NON-PREGNANT FEMALES</u>	<u>POST PARTUM OR PREGNANT FEMALES</u>
Number (%)	1 (20%)	4 (80%)
Age (mean)	64 y/o	(22 y/o)
Survival with Therapy	0	4

It is our contention that young, pregnant females within regions endemic for coccidioidomycosis are at an increased risk for developing *C. immitis* meningitis.

**Abstract 8: Cerebrospinal fluid (CSF) Mycelia (M) and Arthroconidia (A)
in Chronic Coccidioidal Meningitis (Cm)**

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The M of *Coccidioides immitis* has been reported, rarely, in tissue specimens from patients with coccidioidomycosis (C). When it does occur it is usually seen in the setting of chronic cavitary pulmonary disease. We report the unusual occurrence of M of C in the CSF of a patient with chronic CM. A 56 year old Korean male with CM since late 1984 developed hydrocephalus (H) in August 1988 after failing therapy with intra-thecal Amphotericin B (AMB) and miconazole and oral ketoconazole, fluconazole and itraconazole (IZ). He required placement of a lumbar (L) and ventricular (V) Ommaya reservoir (OR) and ventriculo-peritoneal shunt. Over the next 5 months, on IZ therapy, he developed an increased CSF C complement fixation titer, rising CSF leukocyte count in both the L and V fluid, increasing CSF protein and progressive H. He had multiple shunt malfunctions due to obstruction that led to 7 revisions. In January 1989 he grew C. immitis from both OR. Gram stains of spun V CSF revealed M with occasional A. The patient died soon thereafter. We know of only one additional report of M of C in CSF, Meyer et al reported in 1982 a case of a 44 year old male with a 12 year history of CM who developed a recrudescence of disease while on AMB delivered through a Rickham reservoir. He had A seen on 2 specimens drawn from the reservoir, which later grew C. immitis. He died 2 days later. At autopsy M were seen on the catheter leading to the reservoir and also in several pulmonary lesions. Since both this case and our report both demonstrate the occurrence of H in CSF in association with, subcutaneous reservoirs, we suggest that these may provide the correct "micro-environment for the development of C. immitis H in vivo.

**Abstract 9: AN UPDATE ON THE USE OF BAY R 3783 FOR THE
TREATMENT OF COCCIDIOIDOMYCOSIS IN CHIMPANZEES
(PANTROGLODYTES)**

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Six chimpanzees (Pan Troglodytes) were treated for coccidioidomycosis with the experimental triazole, Bay R 3783. The treatment group consisted of five males and one female ranging in age from 4.5 to 28.7 years. Four of the cases were pulmonary infections and the remaining two were disseminated. Diagnosis was based on physical exam findings, Cocci titer, CBC and SMAC results, Westergren sedimentation rates and the existence of appropriate radiographic pulmonary lesions. The dosage rate was 10 mg/kg, once a day. The drug was dissolved in vodka and mixed with fruit flavored drinks. It has been administered orally with good acceptance. The treatment protocol was modified to incorporate treatment interruptions to maintain sufficiently high serum levels of Bay U 3625, the main active metabolite of Bay R 3783. All animals showed marked gross improvement in the first 2-3 weeks of treatment. Although the two animals with disseminated infections have not shown full resolution, continued treatment has minimized the symptoms of the infection with no apparent adverse effects. One animal, whose pulmonary infection was diagnosed and treated early, has shown complete resolution of the infection partially supported by NIH Grant No. RR03602.

Abstract 10: Comparison of four azoles and Amphotericin B in a mouse model of coccidioidal meningoencephalitis

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Prior experience had shown that the triazole Bay R3783 given orally was comparable, at similar dosages, to the triazoles itraconazole and fluconazole in treating coccidioidomycosis induced in the mouse by intranasal inoculation, and superior to ketoconazole. The present evaluation was carried out to determine the efficacy of oral Bay R3783 compared with other azoles and Amphotericin B (AMB) given (i.p.) against coccidioidal meningoencephalitis in the mouse.

Initially intracerebral inoculation with as many as 2,800 endospores of *C. immitis* strain Silveira maintained for many years *in vitro* failed to establish infection. The inoculum was reduced to half or less in the brain in 15 days. Therefore, arthroconidia were utilized. Treatment was begun 2 days after inoculation. In the illustrated experiment, 91 arthroconidia brought about death in all control mice and those treated with ketoconazole 10 mg/Kg b.i.d. At 10 mg/Kg b.i.d., itra, fluco and R3783 only delayed death (itra 100% dead in 35 days, fluco 100% dead in 55 days, R3783 90% dead in 65 days). At 25 mg/Kg b.i.d. all keto treated were dead by day 14, but itra, fluco and R3783 and AMB (1mg/Kg.q.d.) prevented deaths throughout the 3 weeks of therapy. However, deaths occurred in all treatment groups beginning some 8 days (fluco) to 15 days (itra) after cessation of therapy. By day 43 after cessation of therapy, 90% of itra and R3783 mice were dead, and 80% of fluco and AMB mice were dead. Thus, R3783 showed efficacy comparable to fluco, itra and AMB against coccidioidal meningoencephalitis.

Abstract 11: Use of Nikkomycin Z in a mouse model of Coccidioidal Meningoencephalitis

R. Hector, D. Pappagianis and B. L. Zimmer

A mouse model of coccidioidal meningoencephalitis was used. to evaluate the chitin synthase inhibitor nikkomycin Z as an orally administered antifungal agent. Outbred Swiss Webster mice were infected intracranially with 90 arthroconidia of C. immitis, then therapy at 50 mg/kg given b.i.d. was initiated after a 48 h delay. A group was also treated with the azole Bay R 3783 at 25 mg/kg given q.d. Both drugs were given for 21 days, and mice were observed for mortalities over a 65 day period. While there were early deaths in the nikkomycin-treated group, there was 60% survival at the end of the experiment. In contrast, treatment with the azole prevented deaths for the duration of therapy, but then there was a rapid onset of mortality with only a single survival at the end of therapy. Thus, Nikkomycin Z may prove useful in the treatment of this syndrome.

Abstract 12: The Interaction of Azoles with Rifampin (R), Phenytoin, Carbamazepine and Cyclosporin: In Vitro and Clinical Observations

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Twelve patients receiving azole therapy with ketoconazole, itraconazole (IZ) and/or fluconazole (F) for systemic mycoses experienced drug interactions with R, phenytoin and/or carbamazepine resulting in substantial decreases in serum azole concentrations. The reduction seen in F serum concentrations in 1 patient receiving concurrent R was substantially less than that seen in patients receiving IZ and R. Two of 3 patients receiving concurrent cyclosporine and azoles had elevated cyclosporin concentrations associated with concurrent therapy, 1 patient received IZ and I F; there was no apparent effect of cyclosporin upon serum azole concentrations.

Four of 5 patients with cryptococcosis receiving IZ and R responded despite the decreases seen in their serum IZ concentrations. Synergy between IZ and R was documented in vitro against Cryptococcus neoformans isolates from all these patients. In contrast, 2 with coccidioidomycosis failed to respond and 2 suffered a relapse of seborrheic dermatitis while receiving IZ and R. Synergy was demonstrated in vitro for IZ and R against the mycelial phase of coccidioides immitis but not against its pathogenic spherule phase or against Malassezia furfur, the purported etiologic agent of seborrheic dermatitis.

All patients receiving azoles and concurrent therapy with phenytoin and/or carbamazepine failed to respond or suffered a relapse of their fungal infection. These findings illustrate several new clinically significant drug interactions that should be considered in choosing therapy for serious mycoses.

Abstract 13: Effects of Cilofungin on *Coccidioides immitis*

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We studied the effects of cilofungin (LY- 121019), a derivative of echinocandin B, on *C. immitis*. At concentrations of 40 ug/ml or greater, cilofungin blocked mycelial growth. This was paralleled by nearly complete reductions in mycelial incorporation of the chitin precursor, N-acetyl-glucosamine. Decreased GlcNAc uptake presumably reflects broad metabolic inhibition since cilofungin is not known to block chitin synthesis directly. After exposure to cilofungin for 1-5 days, arthroconidia demonstrated blunted conidial growth, vacuolization, collapse of cell walls, and unusual spiral forms. Transmission electron microscopy of similar preparations showed striking defects in the outer wall but comparatively little alteration of internal cell structures. Cilofungin also seemed to slow spherule development. Despite the cilofungin-induced changes in the cell wall, killing of cilofungin-exposed mycelia by human neutrophils was less than 6%. We conclude that cilofungin inhibits growth and alters the morphology of *C. immitis*. Extending these observations to in vivo studies is warranted.

Abstract 14: Activity of Cilofungin in vivo in a murine model of Disseminated Coccidioidomycosis

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The lipopeptide drug, cilofungin, was tested for efficacy in the treatment of a murine model of disseminated coccidioidomycosis. Six week old female Balb/C mice were inoculated intravenously with 210 arthroconidia of Coccidioides immitis. Intraperitoneal therapy was initiated two days post-infection with cilofungin at 62.5, 6.25 or 0.625 mg/kg/day (MKD) in 33% PEG 300 given b.i.d. or Amphotericin B at 6.25 or 0.625 MKD given once daily. Therapy was continued for 19 consecutive days and deaths recorded through 60 days post-infection. Most deaths occurred between days 8 and 24. Only 1/15 untreated control and 0/9 diluent treated mice survived. Survival of mice treated with cilofungin was not prolonged over that of controls, 2/15 treated with 62.5 MKD, 0/14 treated with 6.25 MKD and 1/15 treated with 0.625 MKD of cilofungin survived. 12/15 mice receiving 0.625 MKD and 15/15 given 6.25 MKD of amphotericin B survived through the 60 day period. The residual organ burdens of C. immitis in the spleen, liver and lungs were determined on all survivors. Equivalent burdens were found in the organs of control and cilofungin-treated mice, whereas 6/12 and 15/15 mice treated with 0.625 MKD and 6.25 MKD of amphotericin B, respectively, were free of C. immitis in all organs assayed. Determination of the serum levels of cilofungin after a single dose or 19 days of therapy with 62.5 MKD showed that peak levels occurred by 1 hour postdose at 50 and 53 mcg/ml respectively and dropped rapidly thereafter. In summary, cilofungin exhibited no efficacy in vivo against C. immitis at any dosage tested with no significant improvement over controls as judged by survival or residual organ burden.

