injection of a high dose of radiogold into the same mouse.

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In vitro and *In vivo* Activity of Candicidin on Pathogenic Fungi. (20128)

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Lechevalier, Acker, Corke, Haenseler, and Waksman(1) have established that candicidin is an antifungal agent with activity against certain filamentous and yeast-like fungi. The purpose of the present communication is to define the antifungal spectrum of the water-soluble fraction of candicidin against pathogenic fungi and to estimate its protective effect in mice with systemic mycoses.

Materials and methods. The antibiotic was dissolved in various concentrations in an agar medium containing 2% glucose and 1% neopeptone adjusted to pH 7. A 1:1000 volumetric suspension of the various fungi were streaked over the agar surface. The plates were incubated at 37°C and at room temperature. Estimation of growth inhibition was made within 2 to 3 days after growth was evident in the control plates. This was 2 to 3 days after incubation for rapidly growing yeast-like fungi, such as *Cryptococcus neoformans* and *Candida albicans*, and 7 to 8 days for slower-growing fungi such as *Histoplasma capsulatum* and *Coccidioides immitis*. To determine fungicidal activity a suspension of *C. albicans* in brain heart infusion broth containing approximately one million cells/ml was prepared. The antibiotic was then added in concentrations of 10 and 50 μg/ml. At 2, 4, 6, 8, and 24 hours samples of the broth were removed and the quantity of viable cells determined by plate counts on glucose peptone medium. The heart infusion broth was maintained at 37°C during the test. The final concentration of antibiotic in the media on which the colony counts were made was not sufficient to exert a fungistatic effect.

Experimental infections. Throughout, 20 g white, male mice were used. In some cases injections of the fungi were made intraperitoneally and, in others, intravenously by way of the tail vein. The quantity of injected cells was appropriately regulated so as to avoid overwhelming infection and rapid death. The aim for the most part was to produce sublethal infections of sufficient severity to enable gross comparisons when the animals were sacrificed. The candicidin was administered intraperitoneally in all instances. Generally, it was given for 10 successive doses beginning on the day of infection. The animals were sacrificed on the 14th day.

Results. In vitro antifungal activity. Preliminary studies showed that the antibiotic activity was considerably reduced under acid conditions. At pH 5 and pH 6 approximately 100 times more antibiotic was required for inhibition than at pH 7 and pH 8, in which range the activity was maximal. Human blood slightly diminished the fungistatic power of candicidin. The antifungal activity was slightly greater at room temperature than at 37°C. Comparable results were obtained with broth and with agar streak methods. The antibiotic activity was maintained undiminished in brain heart infusion broth incubated at

TABLE I. Sensitivity of *Candida* Species to Candicidin.

Organism	Strain	Min. conc. for complete inhibition (μg/ml)
C. albicans	12A2	.5
	3	2.5
	4	.5
	5	5
	6	5
C. albicans stellatoidea	12B1	2
	2	5
C. tropicalis	12C1	5
	2	10
	3	2.5
C. pseudotropicalis	12D1	2.5
	2	2.5
C. guilliermondi	12E1	2.5
	2	5
	3	2.5
C. krusei	12F1	5
	2	10
C. parakrusei	12G1	50
	2	25
	3	50

TABLE II. Sensitivity of Pathogenic Fungi to Candicidin.

Organism	Strain	Min. conc. for complete inhibition (μg/ml)
*Blastomyces dermatitidis**	5A2	.5
" " *	5A3	1
*Histoplasma capsulatum**	7A6	1
" " *	7A5	.5
*Paracoccidioides brasiliensis**	8A7	5
Coccidioides immitis	9A4	500
" "	9A5	500
Cryptococcus neoformans	15A2	1
" "	15A5	2.5
*Sporotrichum schenckii**	6A3	200
" " *	6A4	100
Nocardia asteroides	19A1	200
Geotrichum spp.	13A1	2.5
Hormodendrum pedrosoi	4A1	10
Trichophyton mentagrophytes	2A1	500
" *tonsurans*	2C1	500
" *rubrum*	2B1	500
Microsporum canis	1B1	500
" *audouini*	1A1	500
Epidermophyton floccosum	3A2	500

* Yeast phase.

37° C for one week before testing. The drug was thus stable for this period of time.

The sensitivity of various species of *Candida* is shown in Table I. *Candida albicans,* the chief pathogen in this group, was very sensitive. Strain variations were evident. *Candida parakrusei,* which is but rarely a human pathogen, was the most insensitive of this group.

The activity of candicidin against the major pathogenic species is shown in Table II. A unique feature of the antifungal spectrum is the resistance of *Coccidioides immitis* and the ringworm fungi (species of *Trichophyton, Microsporum,* and *Epidermophyton*). This parallels the experience with actidione, another antifungal antibiotic(2). The actinomycete, *Nocardia asteroides,* was resistant. Exclusive of these fungi, candicidin was markedly inhibitory for the major fungus pathogens. When the same plates were examined 2 weeks after the initial reading, there was usually some growth at the minimal concentration which had originally been inhibitory on the first reading. This suggested some degree of deterioration of the antibiotic.

Fungicidal activity. With both 10 and 50 μg of candicidin/ml of the brain heart infusion broth, the *C. albicans* cell count was reduced approximately 45% in 2 hours, 60% in 4 hours, and 90% in 6 hours. Approximately 1% of the cells were viable in 24 hours. The initial colony counts were made on the 2nd day after plating. On the 7th day many more colonies were evident in the same plates. Thus, many of the cells were simply inhibited and not killed.

Toxicity. The LD_{50} for 20 g Carworth Farm white, male mice ranged between 50 and 65 mg kilo for different lots of candicidin. The maximum tolerated daily intraperitoneal dose for a period of 14 days was 0.8 mg/mouse (40 mg kilo). This figure, too, varied for different lots. No irritation was caused by 0.3 ml of a 1% solution placed into the conjunctival sac of rabbits. The 1% solution was not irritating to the human oral mucosa when swabbed over the tongue for one minute every 3 hours for 2 days during the day time. Injection of the 1% solution intradermally and subcutaneously into mice and guinea pigs caused necrosis within 24 hours with the subsequent development of a severe slough. The 0.1% solution was less toxic when injected into the skin of these animals, but moderate necrosis still developed.

Protective effect in experimental infections. Candicidin exerted a marked suppressive effect

TABLE III. Effect of Candicidin on Moniliasis and Torulosis in Mice.

Organism	Strain	No. mice	Infective dose	Treatment	Mortality	Positive* cultures (survivors)	Gross† findings (survivors)	Smears‡
Candida albicans	12A2	46	0.3 ml of 1:50000 suspension intrav.	0.75 mg Candicidin intraper. daily, 10 days beginning day of infection	3/46	1/43	0/43	—
"	3	48			2/48	0/46	0/46	—
"	2	24		0	8/24	16/16	16/16	—
"	3	24		0	6/24	18/18	18/18	—
Cryptococcus neoformans	15A3	18	0.3 ml of 1:1000 suspension intrav.	0.75 mg Candicidin intraper. daily, 10 days beginning day of infection	4/18	14/14	—	14/14 (++)
"	5	24	0.3 ml of 1:10000 suspension intrav.		2/24	22/22	—	22/22 (+)
"	3	18	0.3 ml of 1:1000 suspension intrav.	0	2/18	15/15	—	15/15 (++++)
"	5	12	0.2 ml of 1:10000 suspension intrav.	0	0/12	12/12	—	12/12 (++++)

* Cultures prepared from kidneys of mice with *C. albicans* and from brains of animals inoculated with *C. neoformans*. Animals sacrificed 14th day.

† Gross findings in mouse moniliasis refers to presence of grossly visible kidney lesions.

‡ Smears are India ink preparations made from brains of animals inoculated with *C. neoformans*. The No. of (+) marks refers to relative quantity of capsulated cells seen in smears. The latter are prepared by emulsifying a portion of brain tissue in a few drops of India ink.

on moniliasis in mice, as shown in Table III. The untreated mice exhibited enlarged kidneys with irregular paleness of the surface or with discrete pinpoint white surface lesions. The kidneys of treated mice were rendered sterile and the kidneys grossly were normal. In another experiment not recorded in Table III, the administration of candicidin was delayed until the 4th day after intravenous inoculation in order to allow the infection to become well established. It was then given daily at the rate of 0.75 mg/ day until the 14th day, at which time the sacrificed animals did not show gross lesions, although positive kidney cultures were obtained from 3 of 22 mice. This indicated that candicidin was curative in mouse moniliasis. Candicidin did not have so striking an effect of torulosis of mice (Table III) as it did in moniliasis. Cultures of the brains of treated animals were always positive. Definite suppression was evident, however, as indicated by the diminished number of organisms present in India ink brain smears. When lethal doses of *C. neoformans* were given intravenously, candicidin was not effective in delaying the day of death or in altering the mortality rate. Candicidin exerted a *marked protective effect* on blastomycosis in mice (Table IV). The untreated animals grossly showed numerous granulomatous lesions in their lungs although there was no evidence of infection in the treated group. For the most part the lungs of treated animals were completely free of the organism as indicated by negative cultures. Strain 5A2, which is highly virulent, produced 100% mortality in untreated mice under the conditions of the experiment. Treatment with candicidin prevented this.

Candicidin had a significant protective effect on mice inoculated intraperitoneally with *Histoplasma capsulatum* (Table IV). Strain 7A6 killed 14 of 16 untreated mice in 14 days, whereas only 4 of 24 treated mice died in the same period. Untreated mice which survived showed enlarged spleens. Splenomegaly was present in only a few of the treated mice. Cultures of the spleens of treated animals were positive in about 75% of the survivors, although in most cases only a few colonies were evident as compared to

TABLE IV. Effect of Candicidin on Blastomycosis and Histoplasmosis in Mice.

Organism	Strain	No. mice	Infective dose	Treatment	Mortality	Positive* cultures (survivors)	Gross† findings (survivors)
Blastomyces dermatitidis	5A2	24	0.3 ml of 1:10000 susp. in 5% gastric mucin intraper.	0.75 mg Candicidin intraper. daily, 10 days beginning day of infection	0/24	3/24	0/24
"	3	30			2/30	2/28	0/28
"	2	18		0	18/18	—	—
"	3	12		0	6/12	5/5	6/6
Histoplasma capsulatum	7A6	24	0.5 ml of 1:100 susp. in 5% gastric mucin intraper.	0.75 mg Candicidin intraper. daily, 10 days beginning day of infection	4/24	16/20	2/20
"	8	18			2/18	12/16	3/16
"	6	16		0	14/16	2/2	2/2
"	8	18		0	1/18	15/15	15/15

* Cultures were prepared from lungs of animals with blastomycosis and from spleens of mice with histoplasmosis. Animals sacrificed 14th day.
† Gross findings in mouse blastomycosis refers to grossly visible nodules in lungs, and in mouse histoplasmosis to enlarged spleens.

large numbers recovered from the spleens of untreated animals. When 0.3 ml of 1:100 *H. capsulatum* suspension was inoculated intravenously into 12 mice and treated according to the same dosage schedule, the protective effect of candicidin was not impressive. The spleens of treated animals were uniformly enlarged as much as the control group, although the number of organisms recoverable by culture indicated a lower degree of infection.

Candicidin given at the rate of 0.75 ml daily for 10 days to mice infected with 0.5 ml of 1:100 suspension of *Sporotrichum schenckii* in 5% gastric mucin exerted a pronounced suppressive effect on the disease. The peritoneal surfaces of the intra-abdominal viscera of untreated mice were covered with whitish plaques. Of the 24 treated mice, 6 showed no lesions at all, and the remaining mice exhibited for the most part only a few scattered plaques. This significant suppressive effect should be viewed in relation to the relatively weak *in vitro* effect of candicidin on this organism.

Candicidin administered according to the above dosage scheme was ineffective in protecting 24 mice injected intraperitoneally with a 1:100 suspension of *C. immitis* in 5% gastric mucin.

Discussion. Candicidin is still an impure substance. The different lots available to us varied in antifungal potency and in toxicity. The results must, therefore, be considered as provisional.

The preparations of Candicidin used in these experiments afforded marked protection in moniliasis, blastomycosis, and sporotrichosis of mice. There was a significant but perhaps not strikingly protective effect in histoplasmosis torulosis under the conditions of the experiment. The course of experimental coccidioidomycosis was not affected by this antibiotic.

Candicidin appears to be a promising therapeutic agent because it possesses both fungicidal and fungistatic properties. Little is known about the excretion or destruction of this substance in the body. In its present state of purification, candicidin is too toxic to be injected subcutaneously or intramuscularly. Preliminary studies have shown that it is not absorbed orally. In humans the only possible route of administration would be intravenous infusion of dilute solutions by slow drip.

A potential use of Candicidin would be the prevention of development of a yeast-like fungal flora in the gastrointestinal tract of those receiving such antibiotics as aureomycin, terramycin, and chloramphenicol. Large numbers of organisms belonging to the genus *Candida* become established in the lower gastrointestinal tract of antibiotic-treated humans. In addition, an abundant *Candida* flora arises in the oropharynx of individuals receiving the 3 antibiotics mentioned(3). Certain untoward side reactions are said to be due to this change in the microbial population of the bowel and oropharynx(4). The simultaneous administration of Candicidin with the orally administered antibacterial antibiotics may possibly suppress the development of a *Candida* flora. Our preliminary studies in mice indicate that this is possible. When mice are allowed to imbibe chloramphenicol in their drinking water at the rate of 125 mg/kg for 5 consecutive days, there is a great increase in the yeast flora of the feces. This effect can be entirely prevented by adding 10 mg of Candicidin to the drinking water per day.

Another potential and somewhat unique use for this substance is suggested by the workers at the Communicable Disease Center in Georgia, who have found actidione useful in preventing fungus contamination when trying to isolate organisms such as *C. immitis* and the dermatophytes (the ringworm organisms) (2-5). Saprophytic contaminants, such as *Aspergillus* and *Penicillium,* often interfere with the successful isolation of ringworm fungi. Suppression of these organisms by actidione greatly facilitates isolation. Candicidin would probably have a similar effect.

Summary. The water-soluble fraction of Candicidin has been shown to have strong anti-fungal activity *in vitro* against the major fungus pathogens of man with the exception of *C. immitis* and the ringworm fungi. Candicidin was found to be fungicidal as well as fungistatic. This antibiotic protected mice infected with *C. albicans, B. dermatitidis,* and *S. schenckii.* Candicidin had only a partial

protective effect on torulosis and histoplasmosis of mice.

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Short Persistence of Trichomonas Vaginalis in Reinfected Immune Mice. (20129)

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The chronic tissue infection of mice with *Trichomonas vaginalis*(1) produced by intramuscular injection of the parasites into the hindleg was found(2) to render the animals immune to a reinfection into the leg muscles of the opposite body side. A marked degree of immunity was observed in 70-100% of the animals for periods up to 10 weeks during which the primary lesion persisted. No anatomical lesions and no parasites were found at the site of reinfection in protected animals examined 12 days after the reinoculation. Experiments of this type seemed suitable also to study the fate of the parasites during the early phases of the reinfection. Technically this was facilitated by the strictly localized character of the infection of mice with *T. vaginalis* and because the fate of the parasites could be followed both by microscopical examination and by culture. The results obtained in a group of reinfection experiments are given in this report.

Method. The technic used in the present experiments corresponded closely to that described earlier(2): Adult white mice, from one colony, were infected intramuscularly in the left hindleg with 500,000 parasites contained in 0.5 ml of an overnight culture of *T. vaginalis* in CPLM medium(3). Three to 4 weeks later when, according to earlier experience, a high degree of immunity was present, the immunized mice were reinfected with the same dose of *T. vaginalis* into the muscles of the right hindleg. A few drops of sterile India Ink were added to the culture dilution in order to mark the site of the infection in the tissues. Groups of normal mice received the same infection. At different intervals after the infection, 4 hours, 8 hours, 1 day, 3, 6, 7, 8, and 10 days, groups of 5 to 10 immune and normal mice were sacrificed and the site of the infection was examined microscopically for the presence or absence of motile trichomonads. Cultures in CPLM medium were taken at the same time from the tissues of both the immune and normal groups and examined after 48 hours incubation. The primary lesions of the immunized mice were also examined.

Experimental. A series of 5 experiments, all giving comparable results, was carried out. The data, which are given in Table I, are based on 2 experiments for the intervals of 4 hours and 1 day and on 3-4 experiments for the remaining intervals of 8 hours, 3, 6-8, and 10 days.

All mice of the immune groups were carriers of large abscesses at the site of the primary immunizing infection. The pus of these abscesses contained numerous active parasites which grew abundantly in culture. The fate of the parasites of the reinfection is indicated by the outcome of the cultures taken at the different intervals after the reinfection. In groups no. 2 to 6, covering the intervals from 8 hours to 10 days, the majority of the immune mice did not contain viable parasites at the site of the reinfection. This is demonstrated by the fact that 70-100% of the cul-